1D and 2D experiments set up by NMR

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GRC NMR Core Facility

Experiments set up in GRC

- 1D ^{1}H NMR
- 1D ^{13}C NMR
- 2D homonuclear NMR
- 2D heteronuclear NMR
- Measurement of coupling constants
- DOSY

1. 1D ^{1}H NMR

- **^{1}H NMR (一般氫光譜實驗)**
  - 1GRC_1D_1H
  - 1GRC_1D_1H-homodecouple

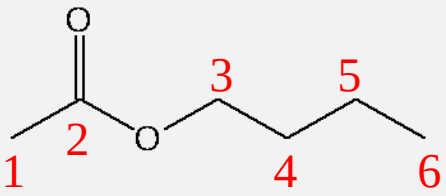
- **Solvent Suppression (溶劑峰壓制實驗)**
  - 1GRC_1D_1H-zgpr
  - 1GRC_1D_1H-zgcppr
  - Multiple Solvent Suppression

- **Selective Excitation (選擇性激發實驗)**
  - sel_COSY
  - sel_TOCSY
  - sel_NOESY → Button NMR set up
  - sel_ROESY

1.1 ^{1}H NMR

IGRC_1D_1H

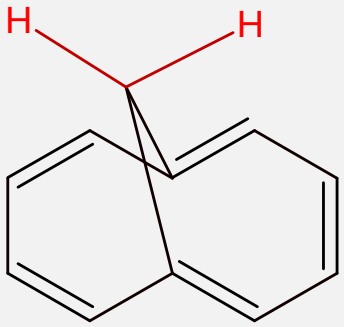
^{1}H NMR光譜



n-butyl acetate

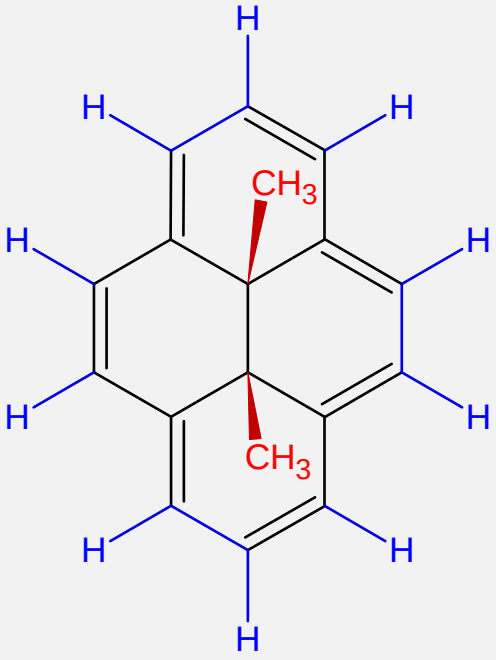
1.1 .1 ^{1}H NMR

- **Anisotropy effect**



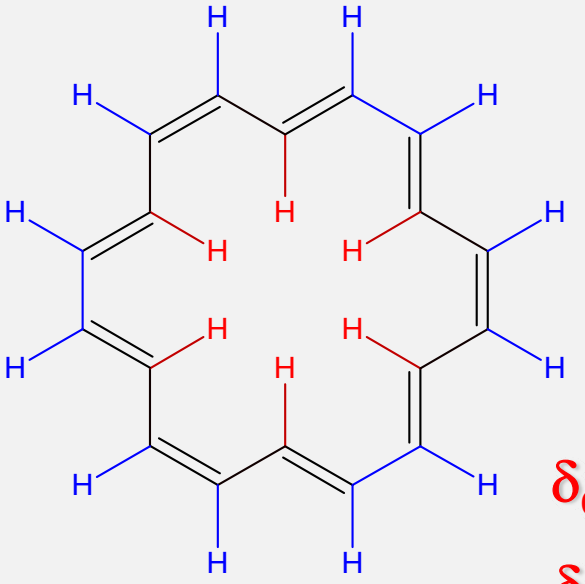
δ_{ring} → 7.27-6.95 ppm

δ_{Me} → -0.51 ppm



δ_{ring} → 8.14-8.64 ppm

δ_{Me} → -4.25 ppm



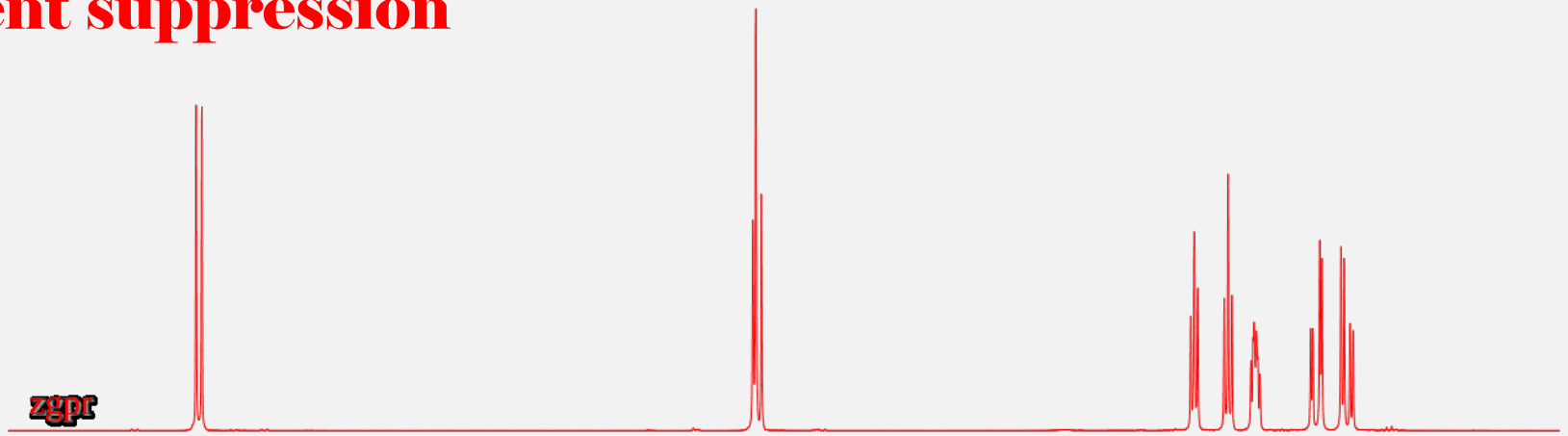
$\delta_{OUTSIDE}$ → 9.28 ppm

δ_{INSIDE} → -2.99 ppm

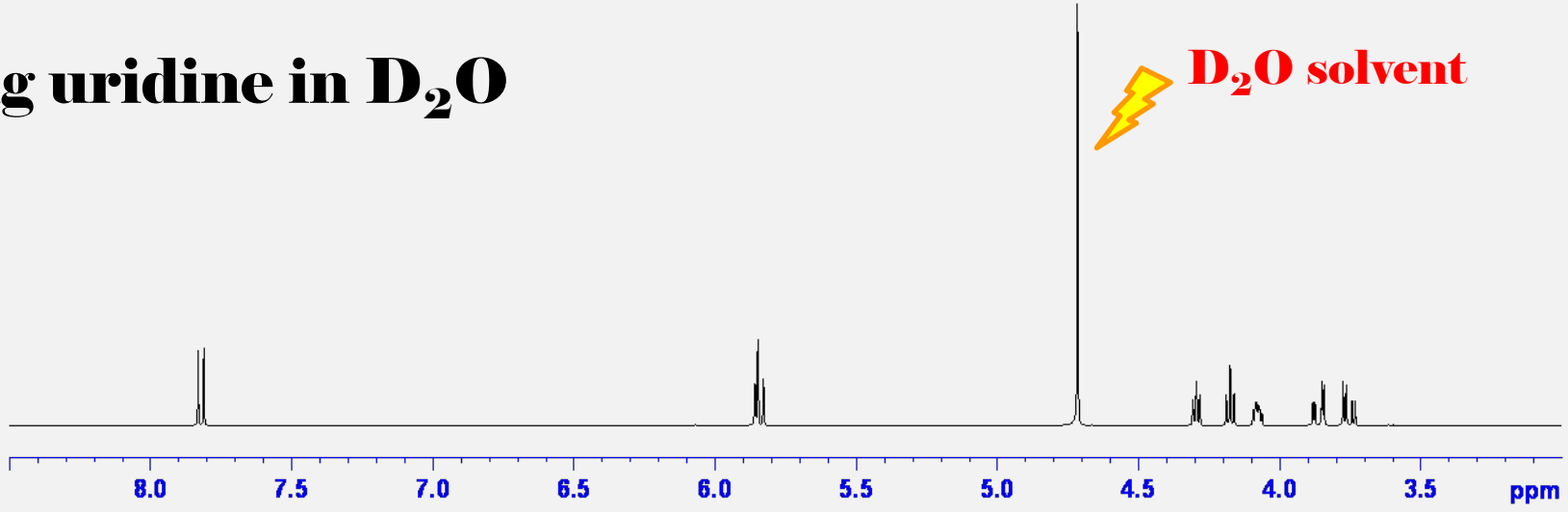
1.2 Solvent Suppression

1GRC_1D_1H-zgpr

solvent suppression



25mg uridine in D_2O



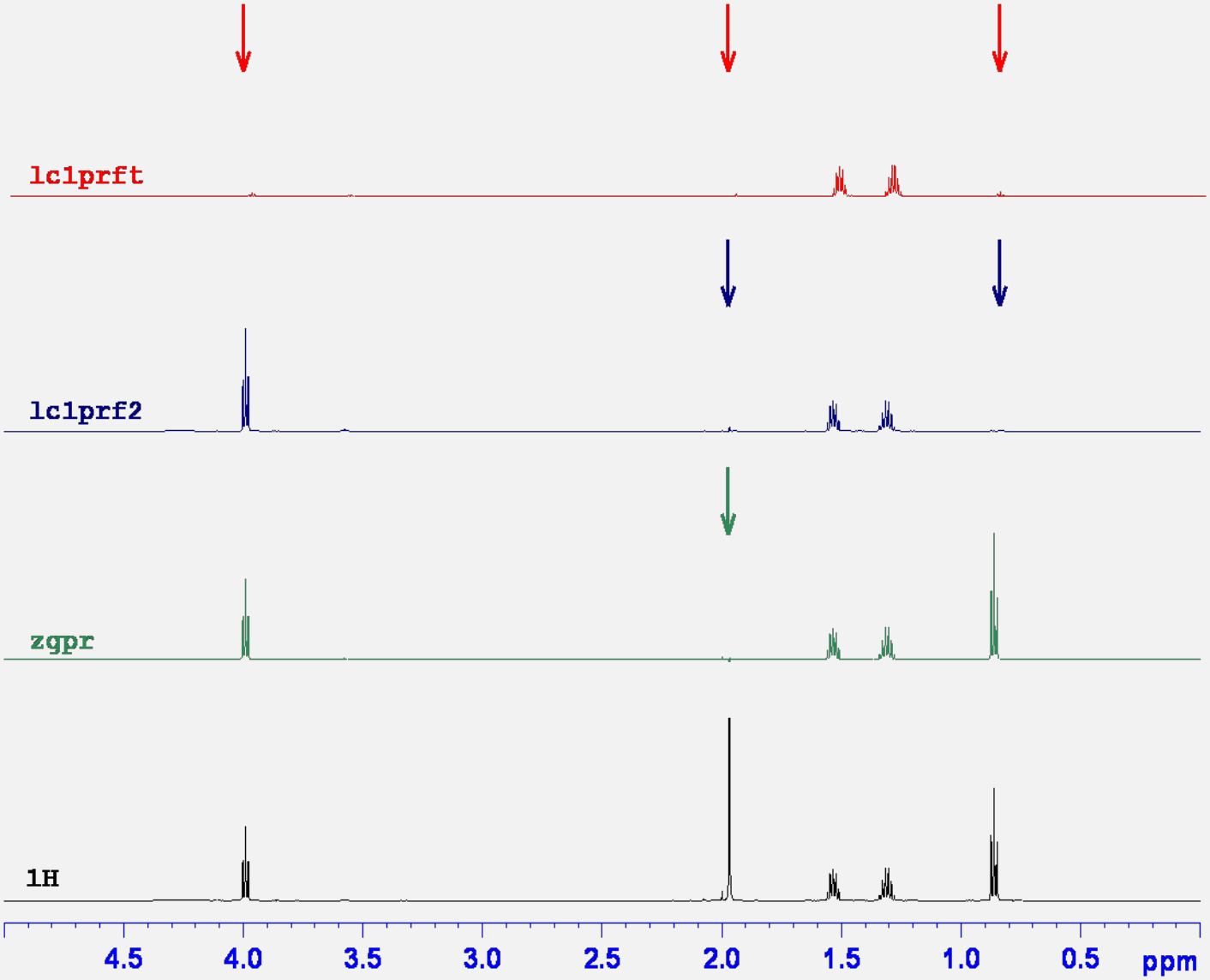
1.3 Multiple Solvent Suppression

- 利用不同的頻道進行溶劑訊號之壓制(一般實驗)
  - lclprf2(2), lclprft(3), lclpnf2(2), lclpnft(3), lclpncwfd(2) (括號內為壓制溶劑訊號之數目)

- 利用shape pulse進行溶劑訊號之壓制(PS系列)
  - zgps, lclpnps, lclpncwps

- 利用WET scheme進行溶劑訊號之壓制(WET系列)
  - wet, wetdc, wetdw

Multiple Solvent Suppression

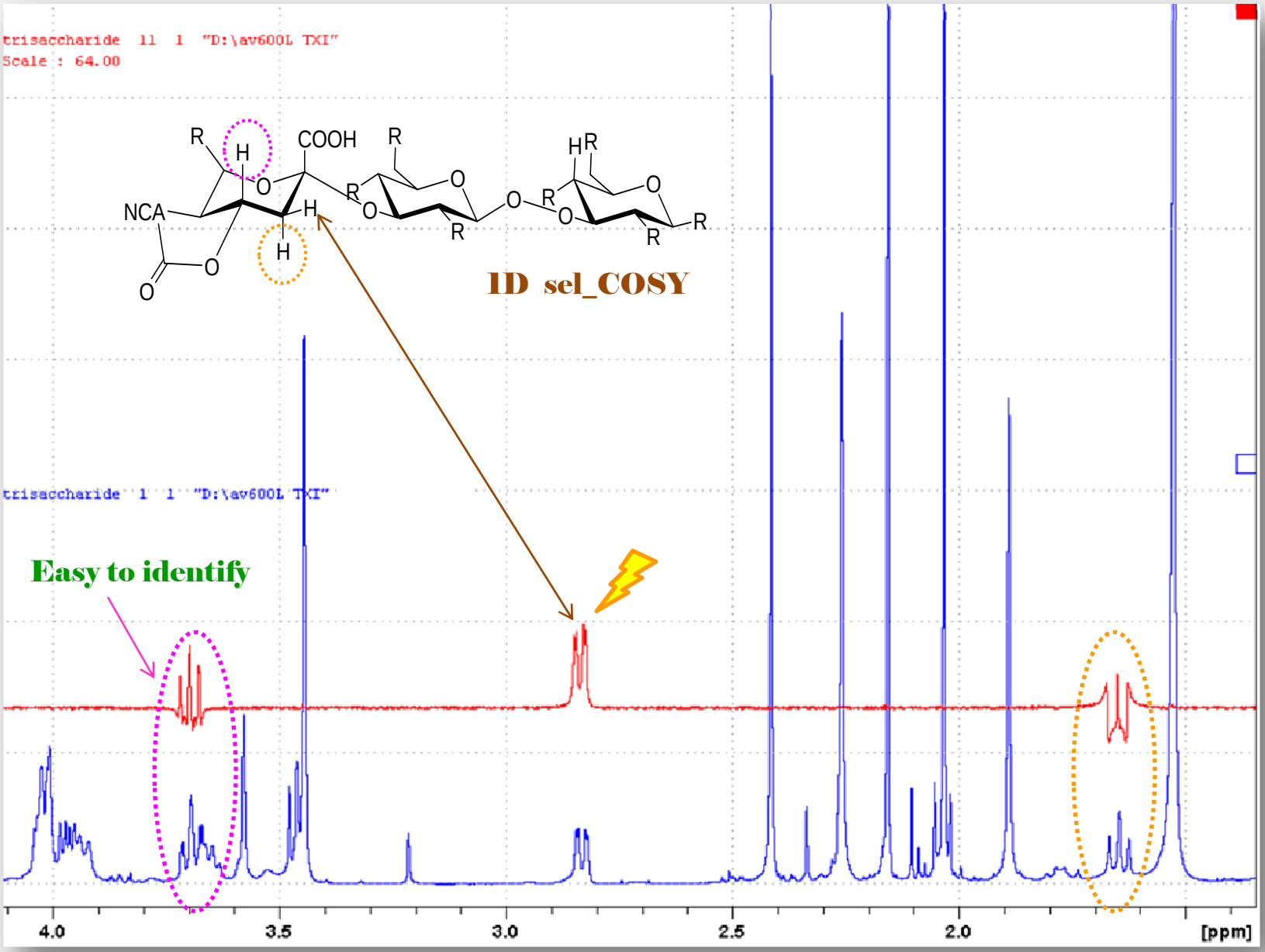


1.5 1D Selective Excitation

- COSY, TOCSY, NOESY, ROESY四種實驗
- 針對特定peak，觀察其相關性
- 減少實驗維度、將目標凸顯、簡化問題、減少實驗時間
- 方便低濃度樣品進行測定
- 可使用Button selective NMR軟體進行實驗

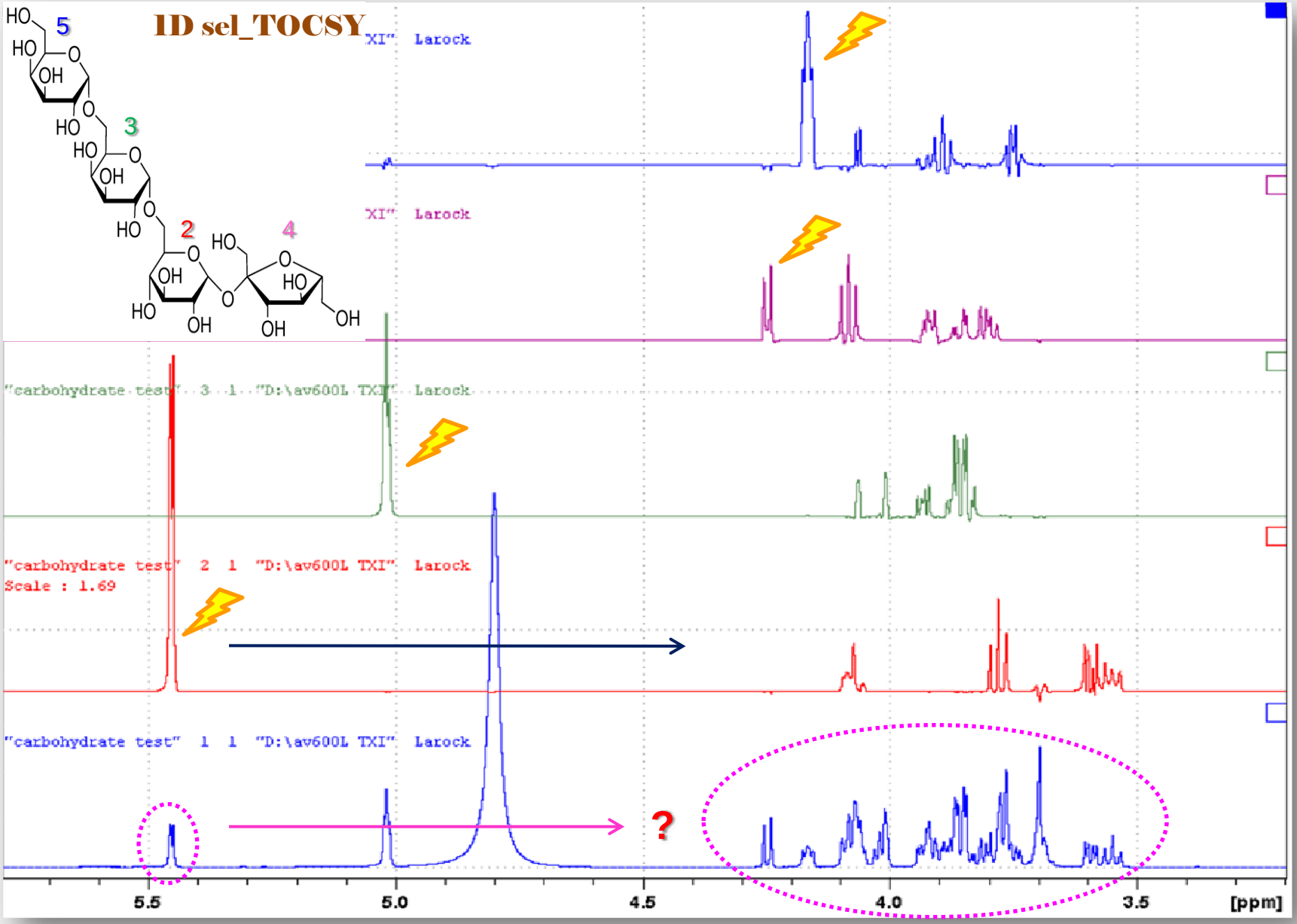
1.5.1 1D Selective COSY

Button NMR set up



1.5.2 1D Selective TOCSY

Button NMR set up



2. 1D ^{13}C NMR

- **^{13}C NMR (一般碳光譜實驗)**
  - 1GRC_1D_13C
  - 1GRC_1D_13C-without decouple
  - 1GRC_1D_13Cint

- **Distortionless Enhancement by Polarization Transfer (區分碳的級數)**
  - 1GRC_1D_DPET45
  - 1GRC_1D_DEPT90
  - 1GRC_1D_DEPT135

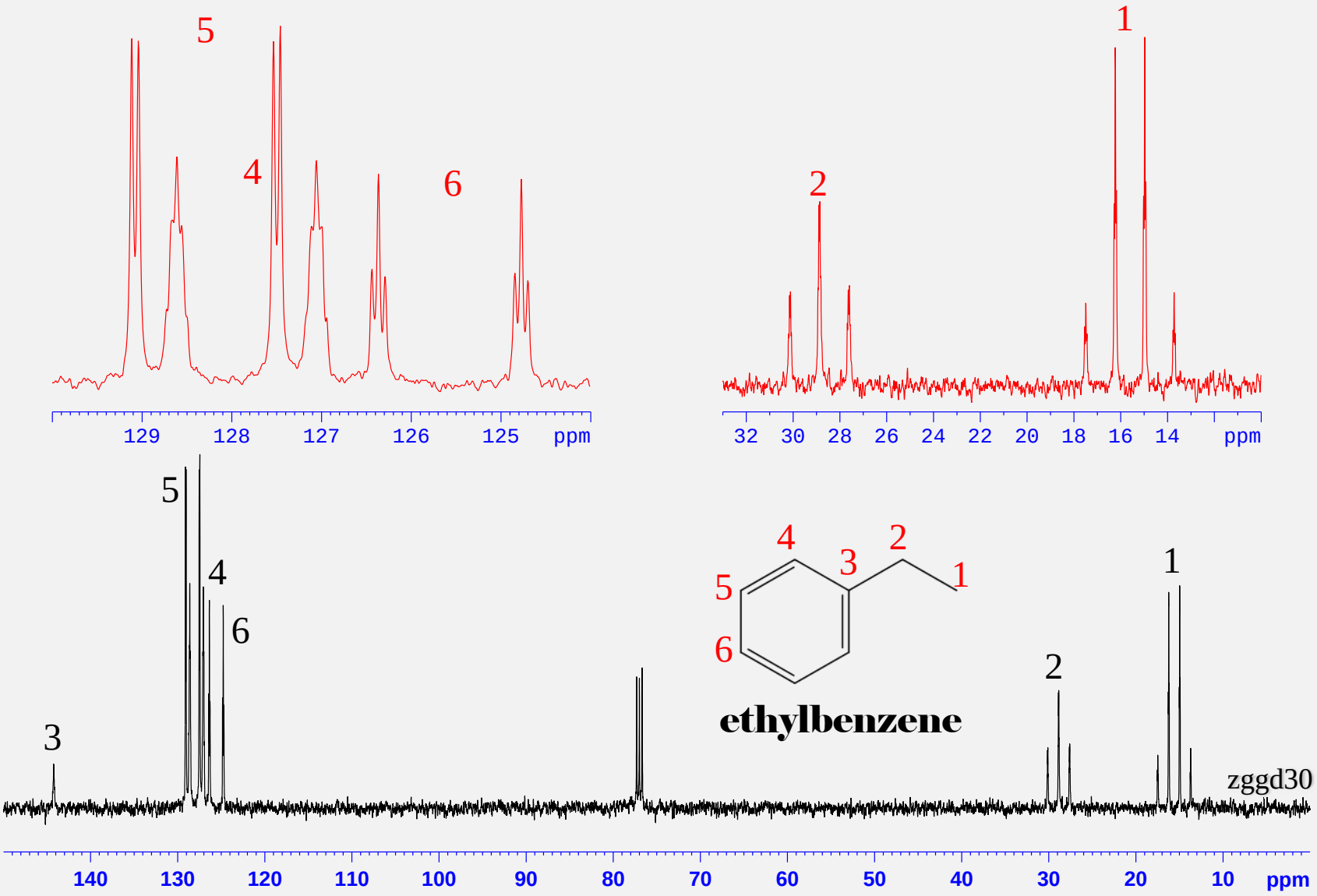
2.1 ^{13}C NMR

^{13}C光譜特性

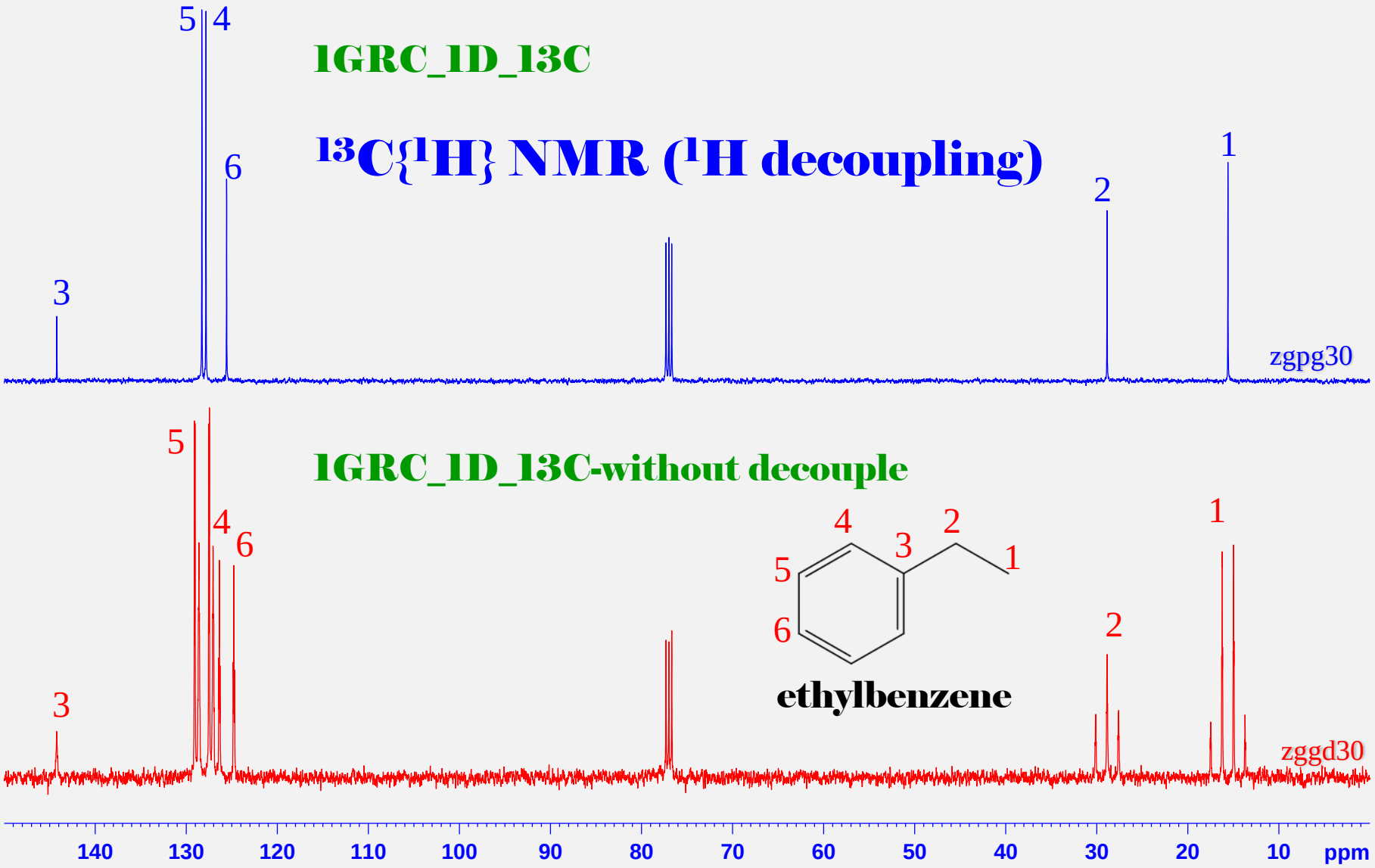
- 因^{13}C 自然含量低，則^{13}C -^{13}C彼此間偶合可忽略
- ^{13}C會受到周圍氫原子的影響而產生分裂
- 其分裂較為複雜，而造成光譜難以判斷
- 受到直接接於碳上的氫原子之分裂較為明顯，並符合2NI+1規則
- 訊號亦會因分裂造成強度下降,一般以去偶合(decoupling)方式進行實驗

2.2 自旋-自旋分裂之^{13}C NMR

1GRC_1D_13C-without decouple



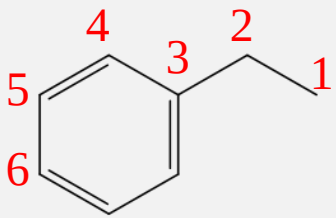
2.3 $^{13}C\{^1H\}$ NMR


5
4
6
3
2
1
1GRC_1D_13C
$^{13}C\{^1H\}$ NMR (^1H decoupling)
zgpg30
5
4
6
3
1GRC_1D_13C-without decouple
4
2
3
1
5
6
ethylbenzene
2
1
zggd30
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

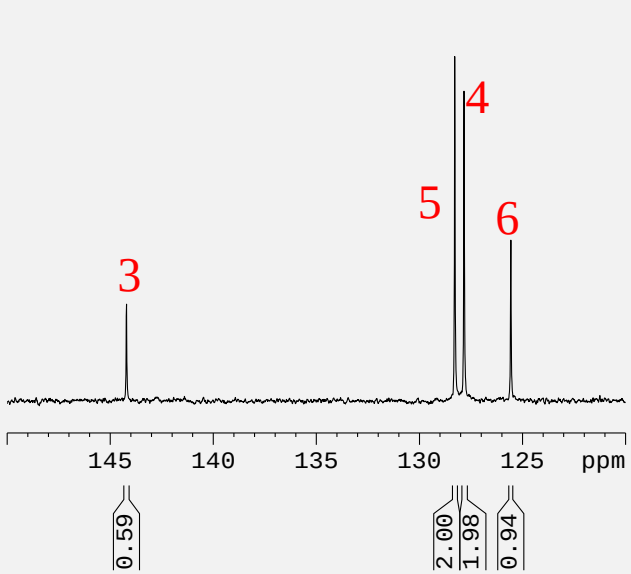
2.4 Inverse Gated Decoupling

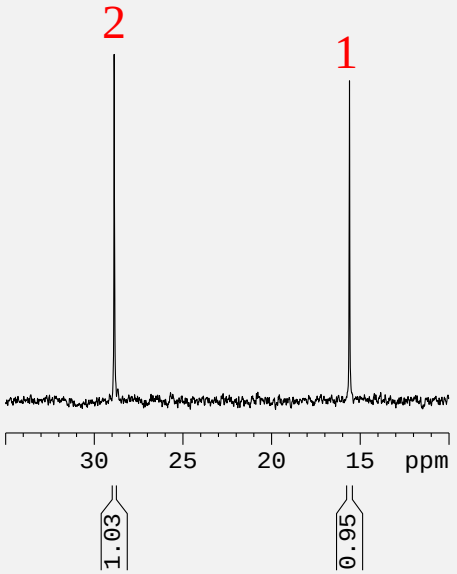
IGRC_1D_13Cint

獲得積分值



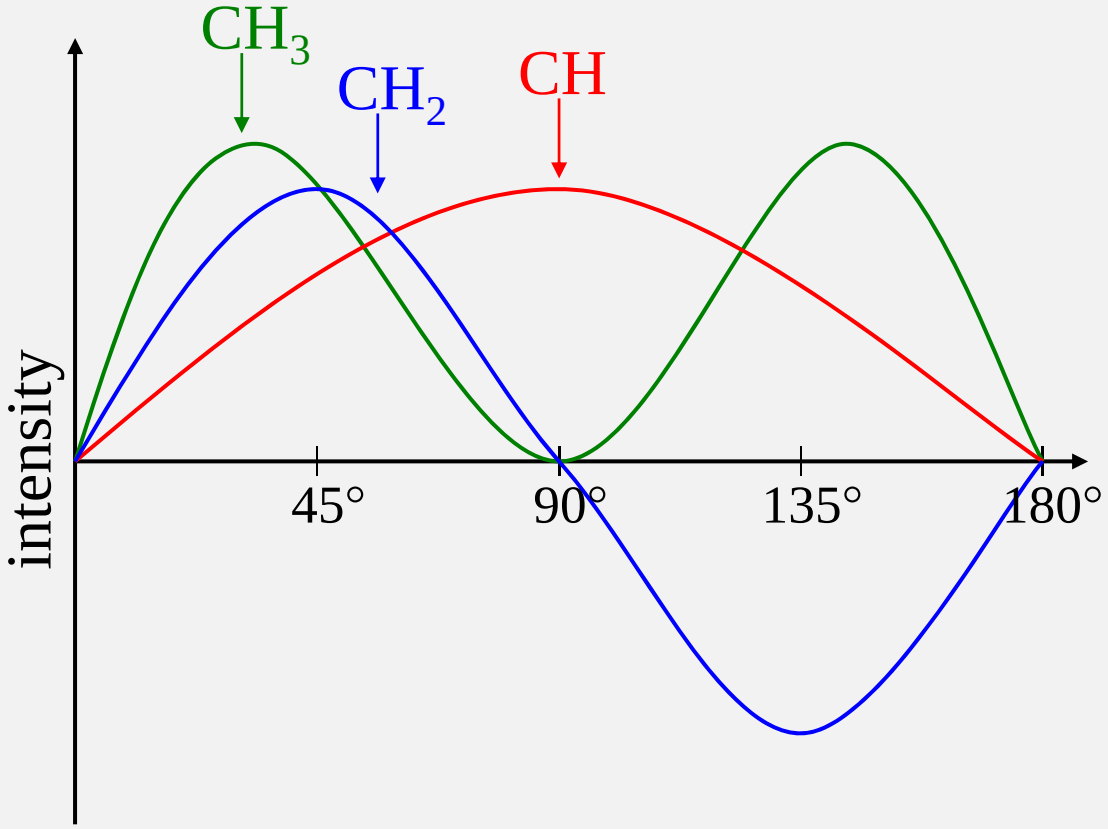
ethylbenzene





2.5.1 DEPT

Distortionless Enhancement by Polarization Transfer



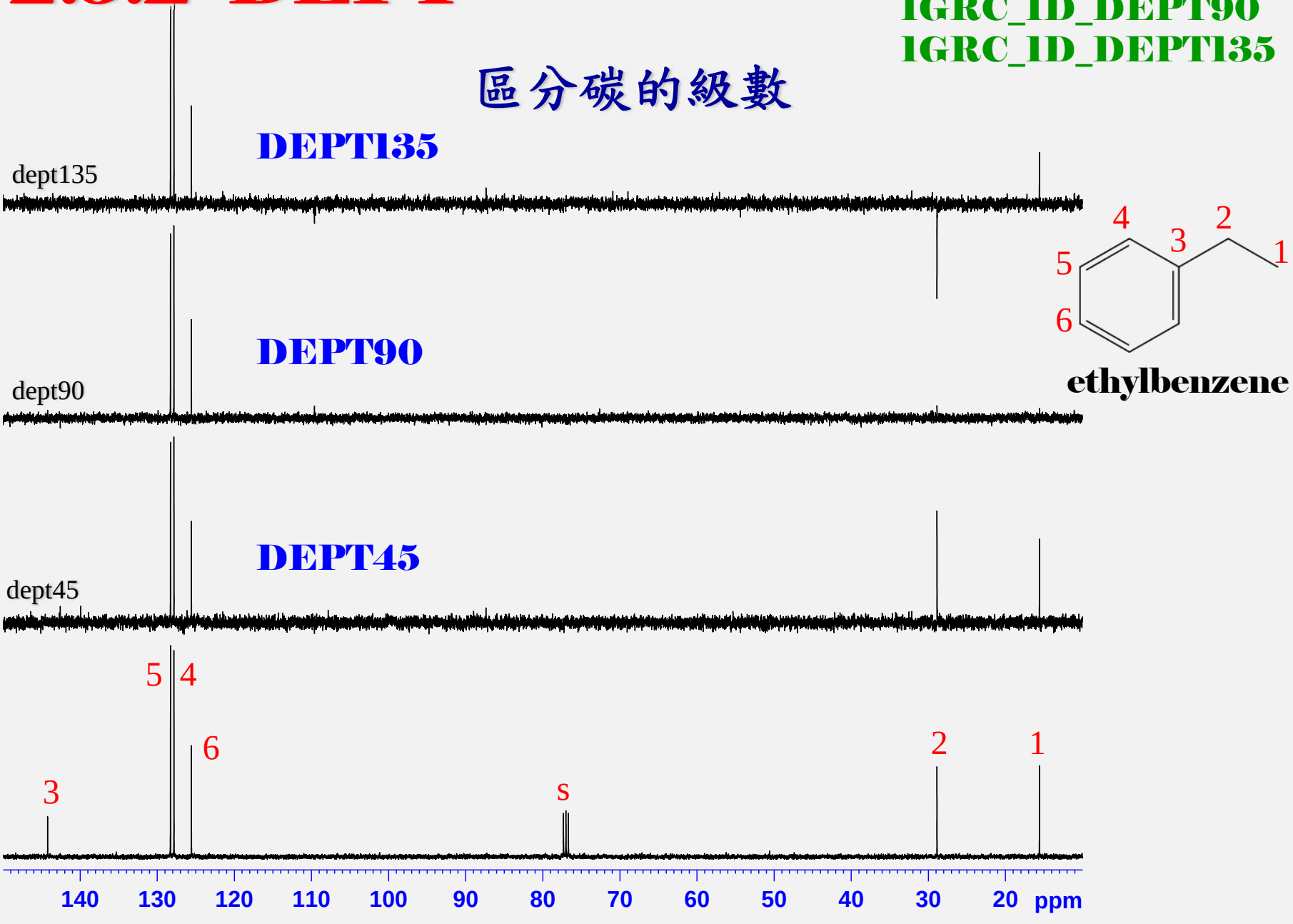
IGRC_1D_DEPT45
IGRC_1D_DEPT90
IGRC_1D_DEPT135

	45°	90°	135°
CH	+	+	+
CH_2	+	×	-
CH_3	+	×	+

2.5.2 DEPT

IGRC_1D_DEPT45
IGRC_1D_DEPT90
IGRC_1D_DEPT135

區分碳的級數



ethylbenzene

3. 2D homonuclear NMR

COSY

1GRC_2D_COSY45
1GRC_2D_COSY90
1GRC_2D_COSY-suppression

DQF-COSY

1GRC_2D_COSY-DQF
1GRC_2D_COSY-DQF-suppressin

TOCSY

1GRC_2D_TOCSY_dipsi
1GRC_2D_TOCSY-mlev
1GRC_2D_TOCSY-mlevg
1GRC_2D_TOCSY-suppression

NOESY

1GRC_2D_NOESY
1GRC_2D_NOESYg
1GRC_2D_NOESY-suppression

ROESY

1GRC_2D_ROESY
1GRC_2D_ROESYg
1GRC_2D_ROESY-suppression

INADEQUATE

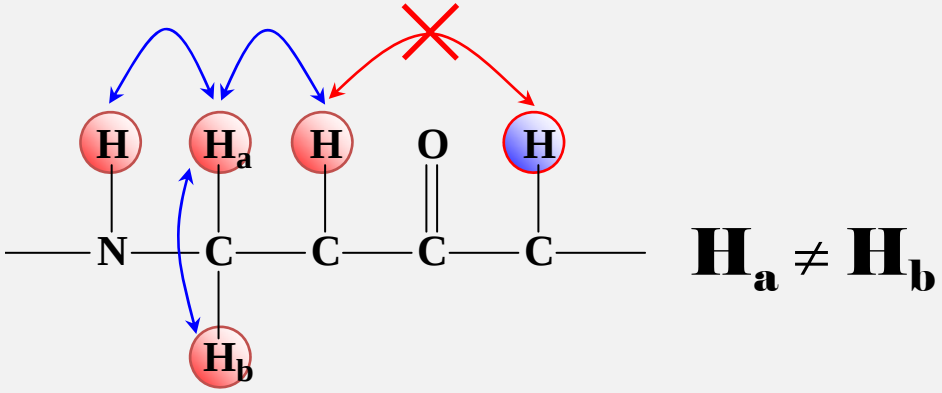
1GRC_2D_INADEQUATE

3.1 2D homonuclear COSY

- **COSY**

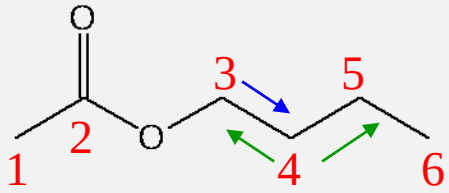
  (Correlation Spectroscopy)

  獲得^{2}J和^{3}J之關係

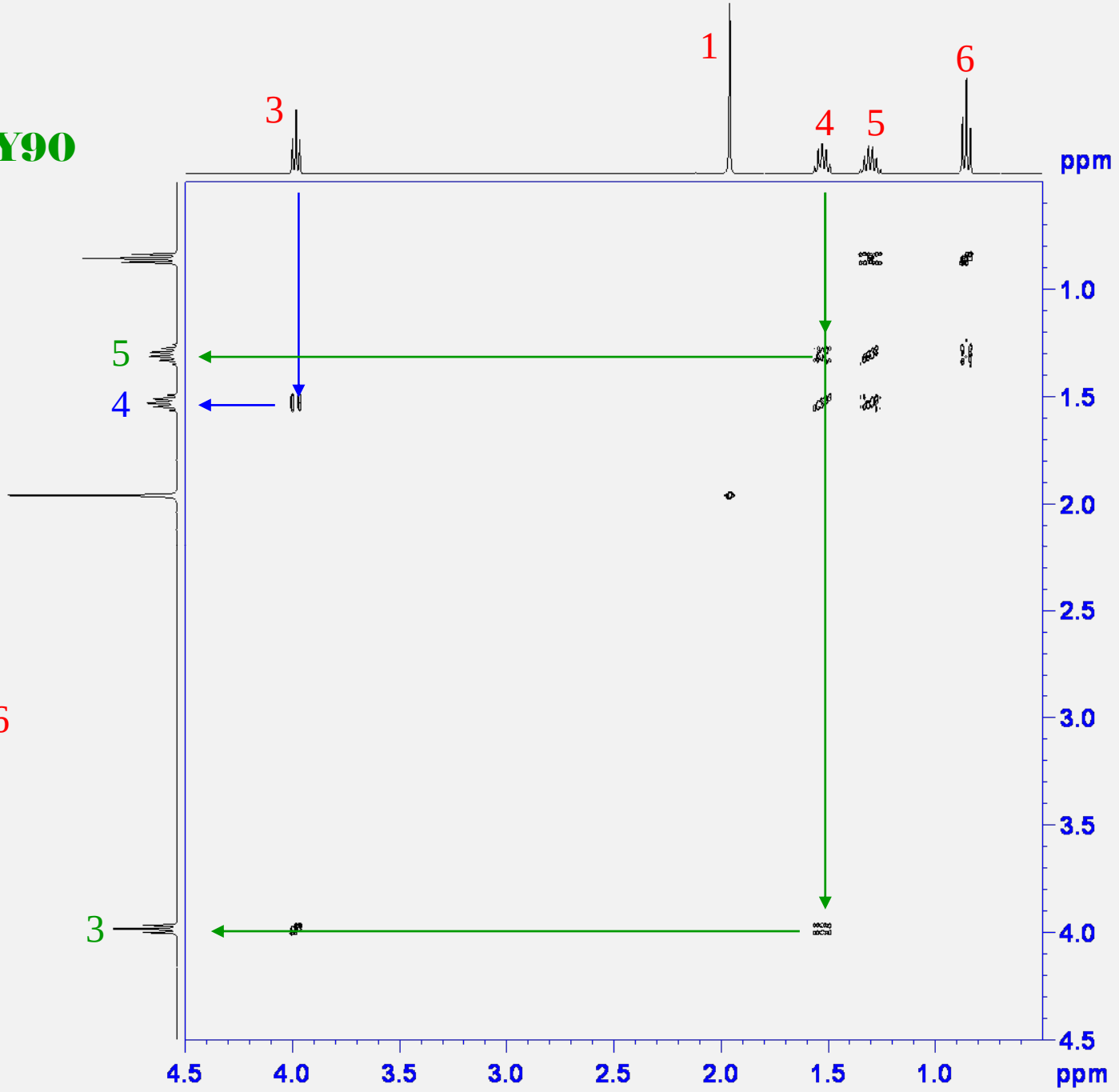


COSY

IGRC_2D_COSY90

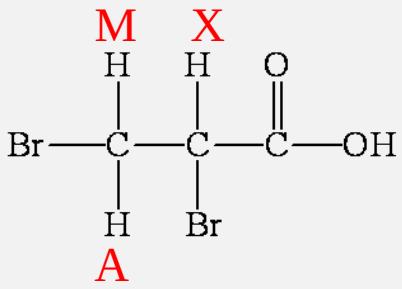


n-butyl acetate

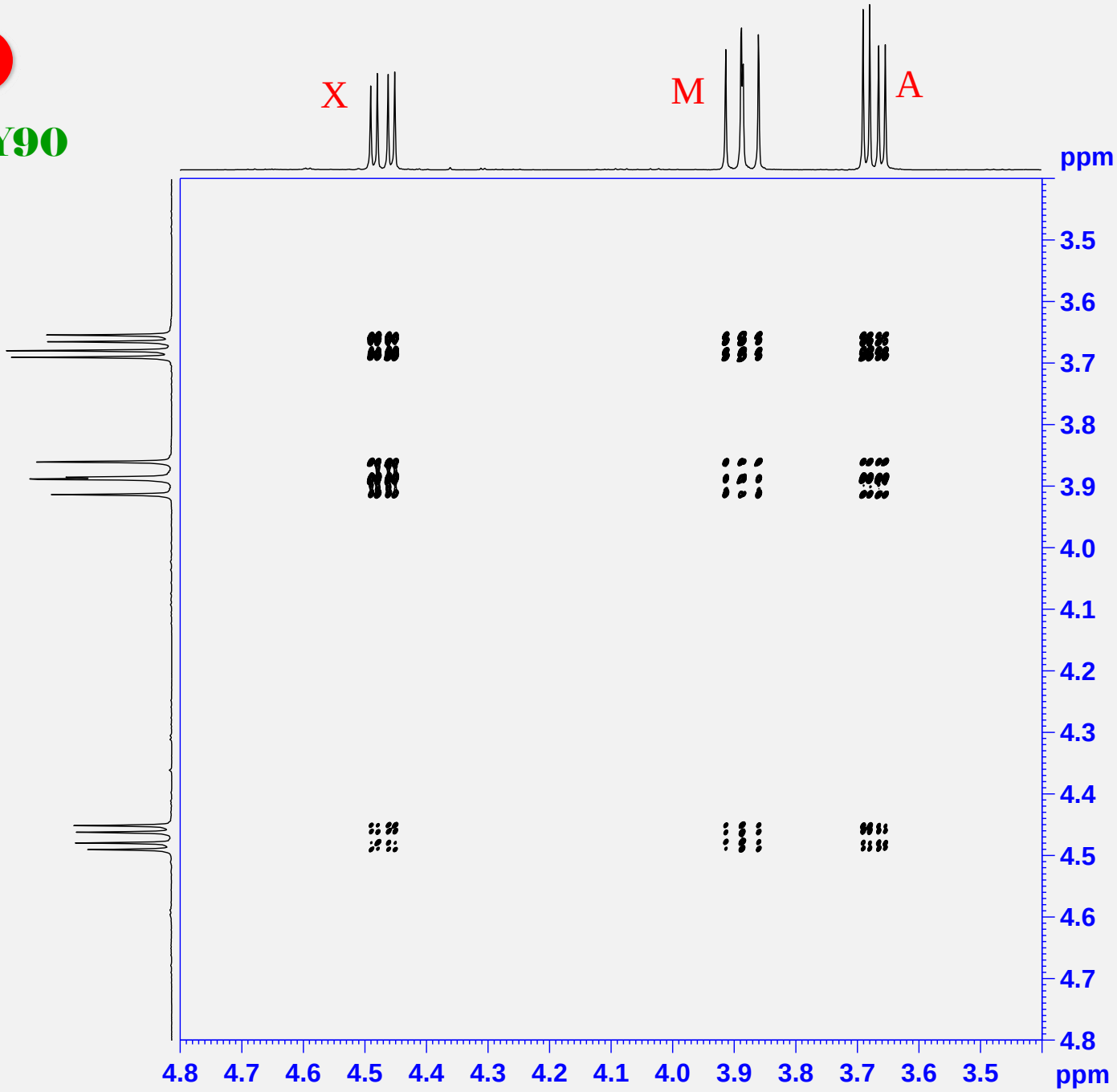


COSY90

IGRC_2D_COSY90



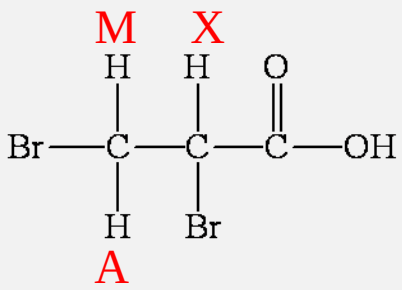
2,3-dibromo-propionic acid



COSY45

IGRC_2D_COSY45

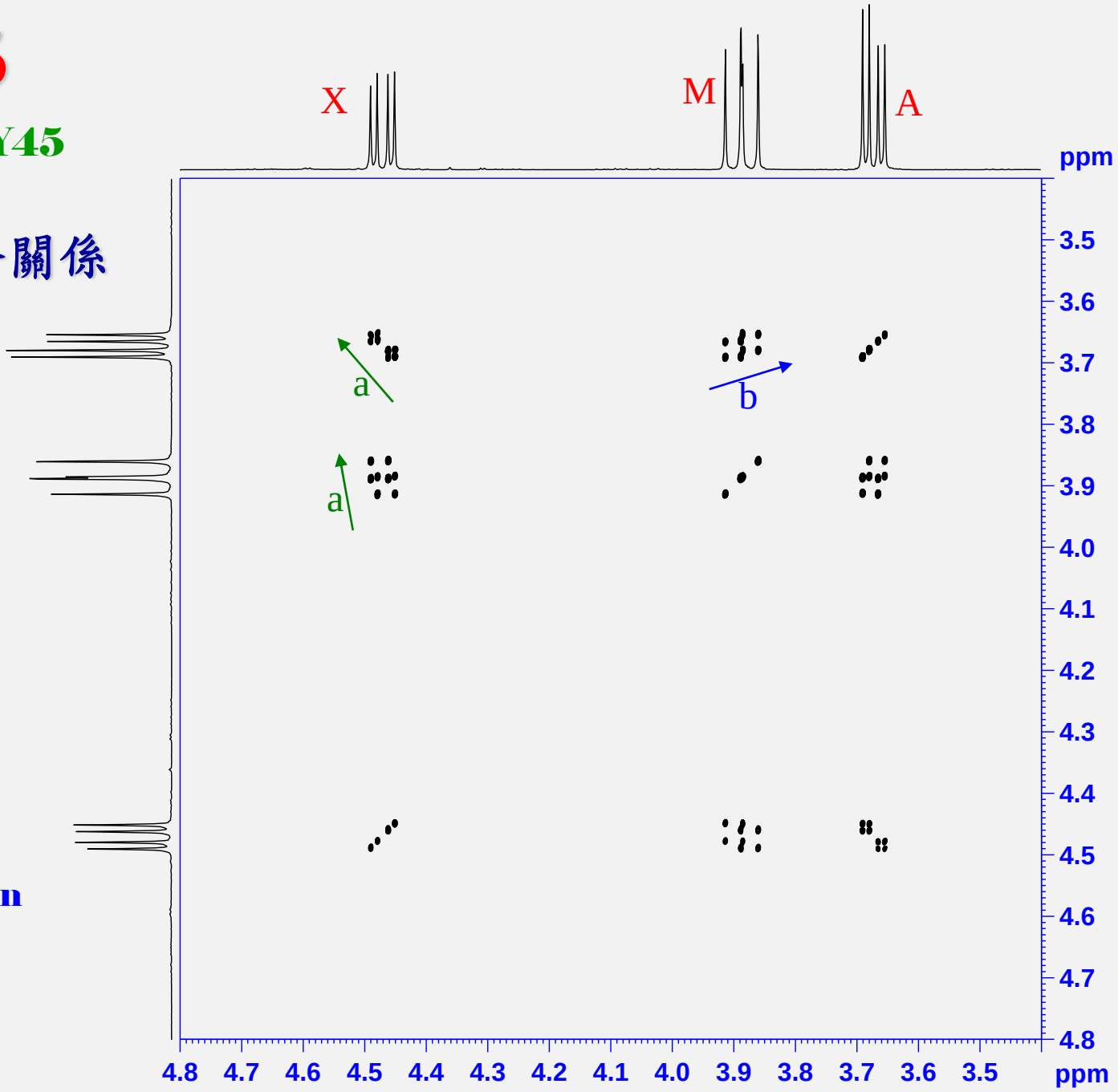
區分^{3}J與^{2}J偶合關係



2,3-dibromo-propionic acid

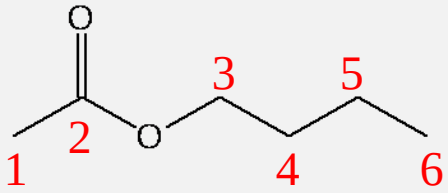
(a)vicinal spin-spin coupling (^{3}J)

(b)geminal spin-spin coupling (^{2}J)



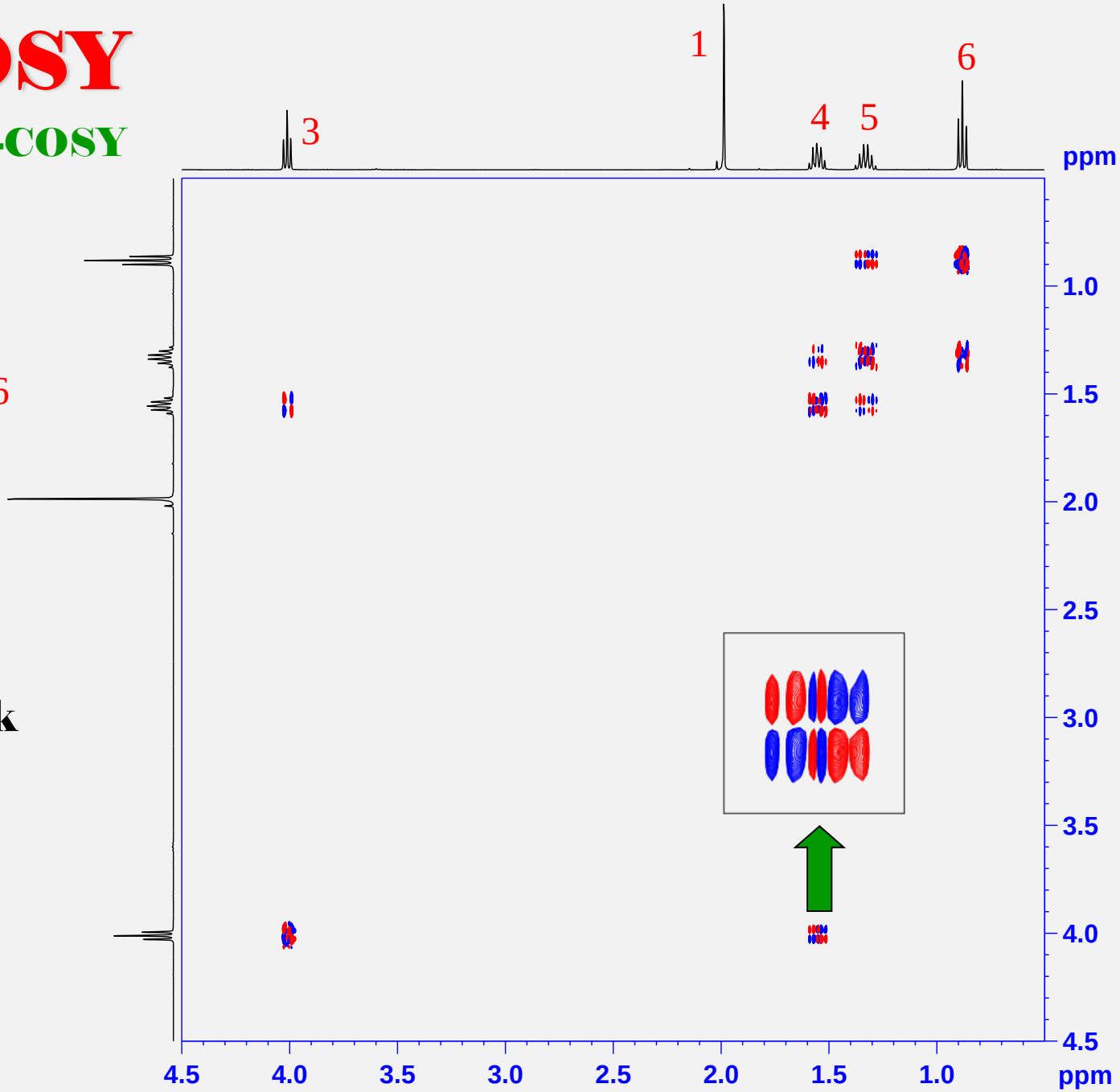
DQF-COSY

IGRC_2D_DQF-COSY



n-butyl acetate

color of the peak
positive peak
negative peak

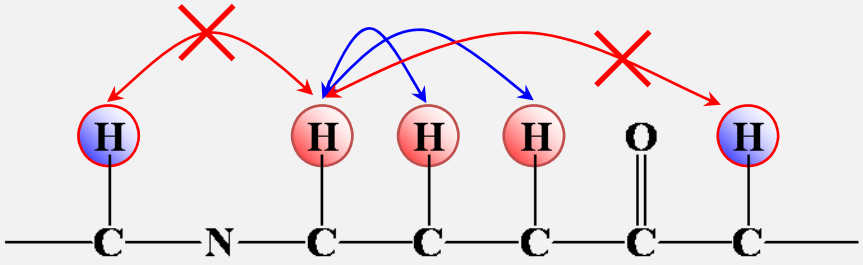


3.2 2D homonuclear TOCSY

- **TOCSY**

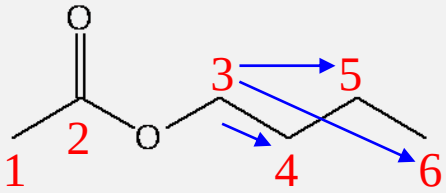
  (Total Correlation Spectroscopy)

  獲得所有J偶合關係

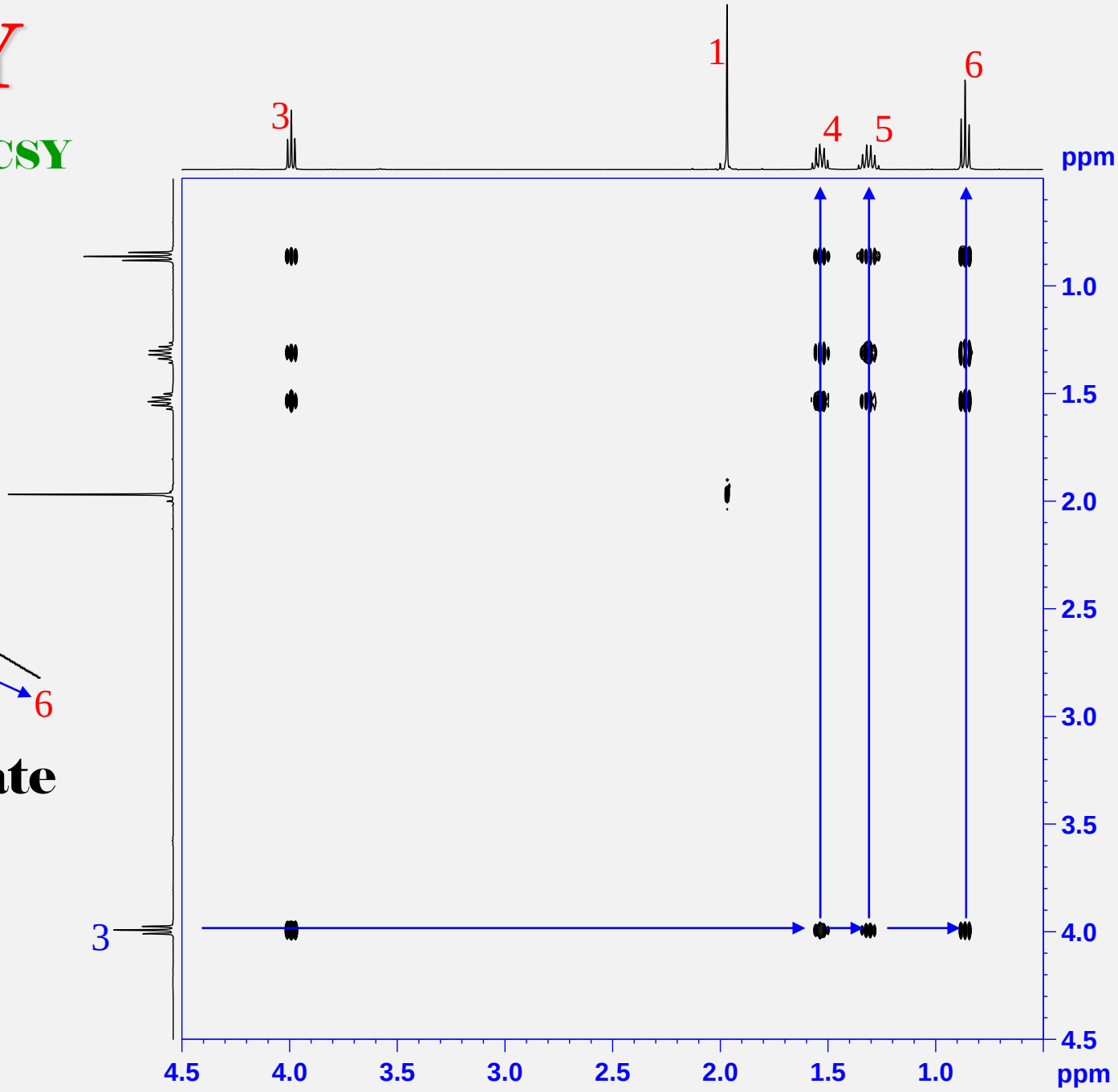


TOCSY

IGRC_2D_TOCSY



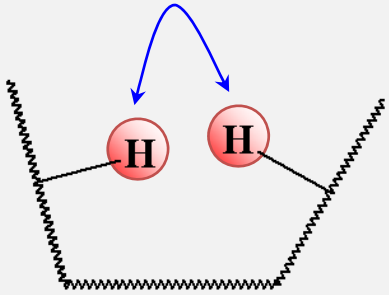
n-butyl acetate



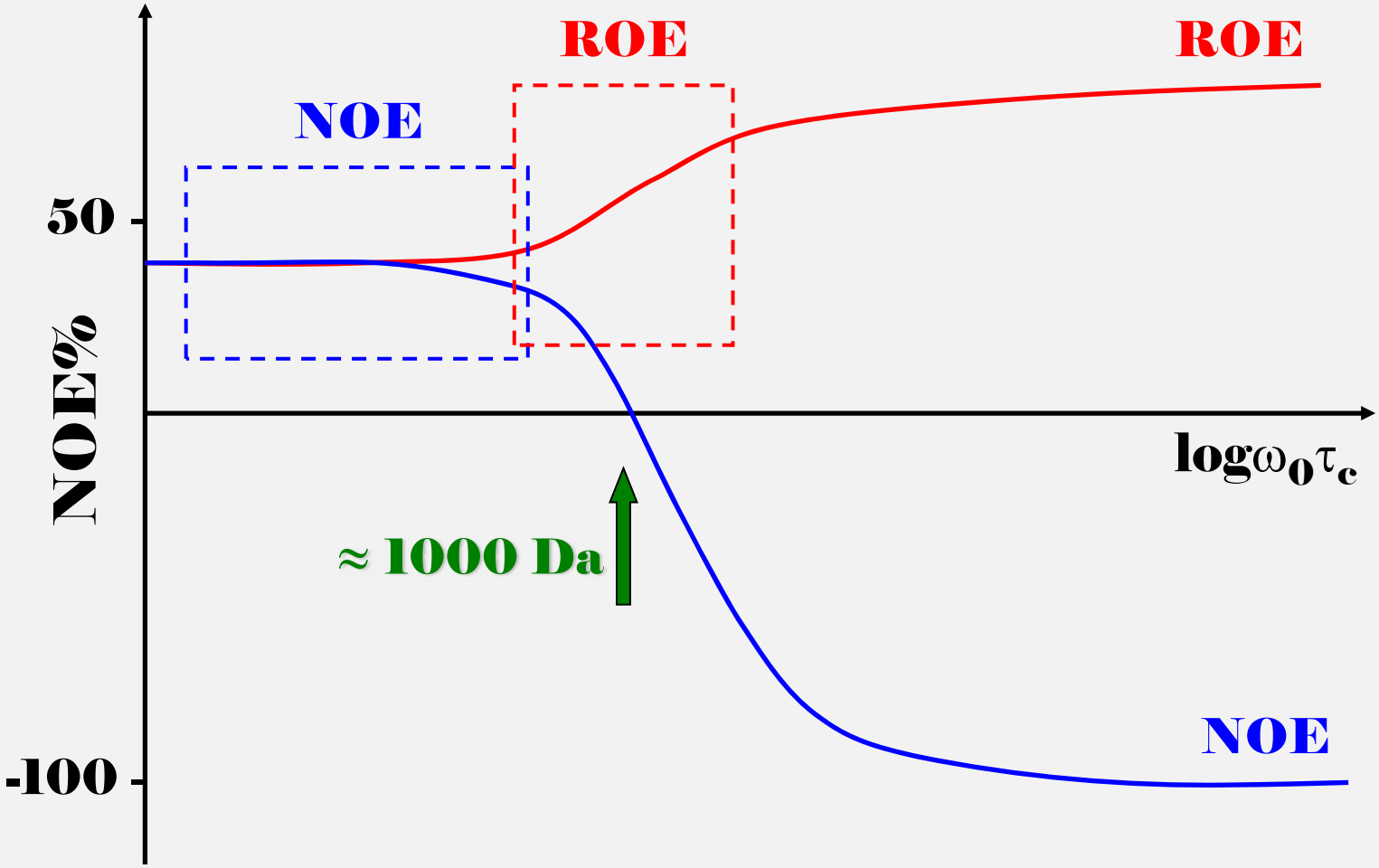
3.3 2D homonuclear NOESY/ROESY

- **NOESY**
  (**N**uclear **O**verhauser **E**ffect **S**pectroscop**y**)

- **ROESY**
  (**R**otating-frame **O**verhauser **E**ffect **S**pectroscop**Y**)
  獲得空間中之關係, < 5Å

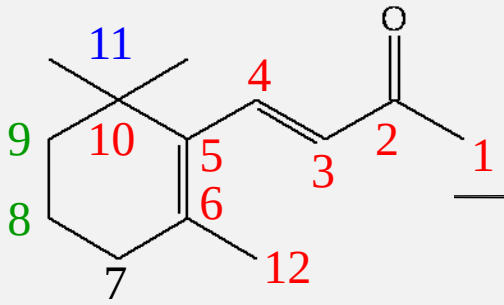


NOESY與ROESY



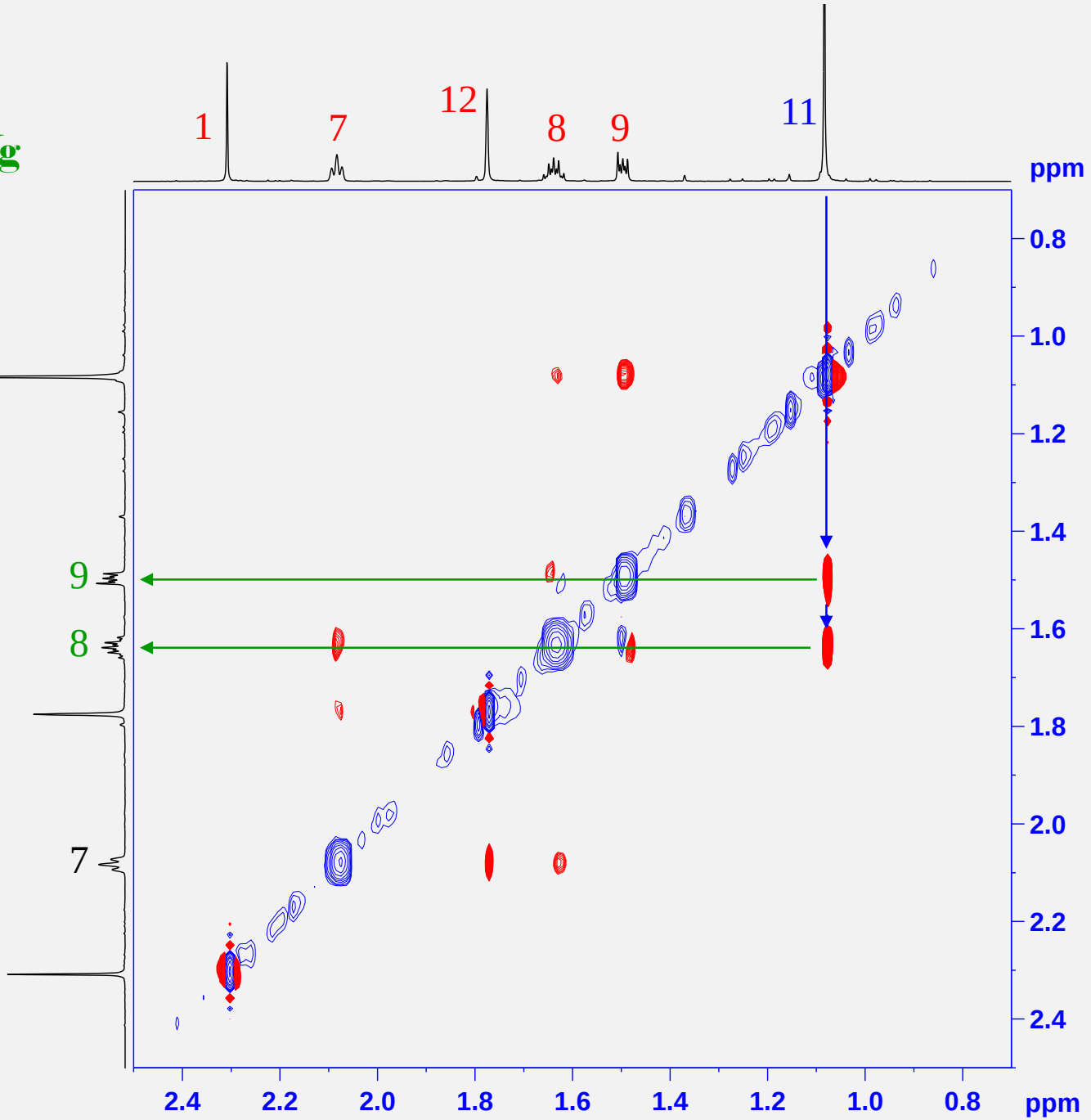
NOESY

IGRC_2D_NOESYg



β-ionone

color of the peak
positive peak
negative peak

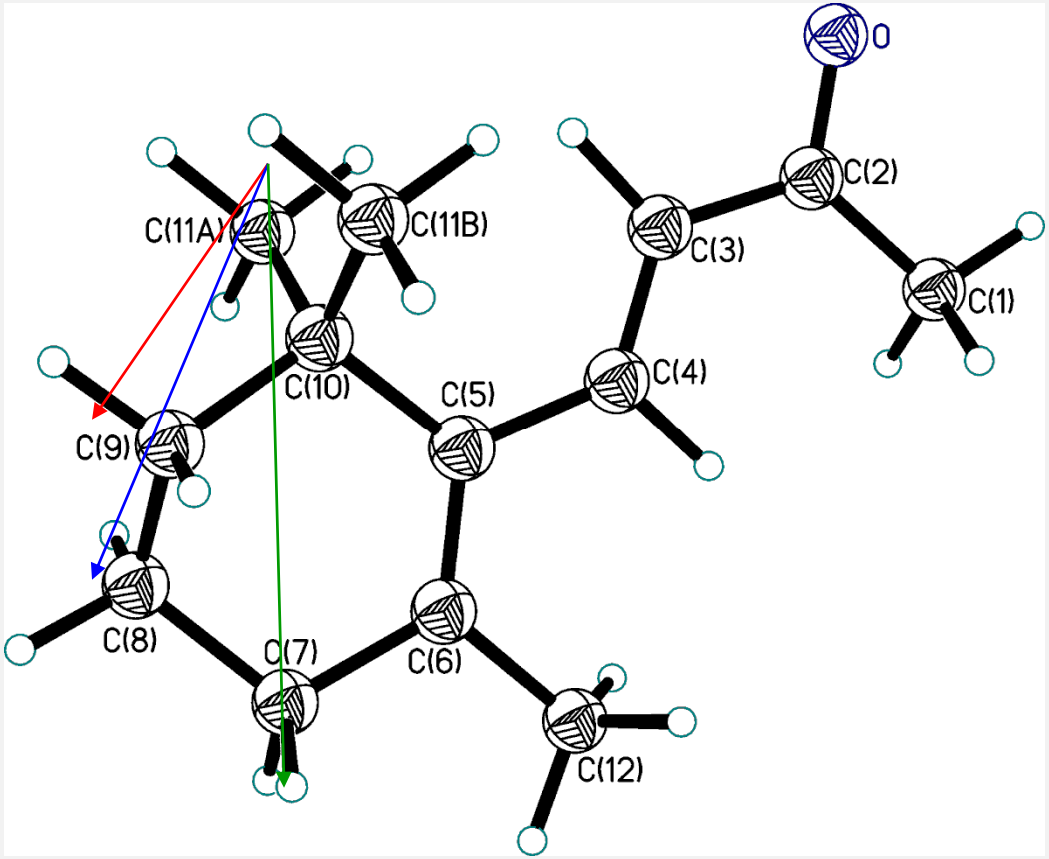


NOESY

C(11)上的H – C(9)上的H
≈ 2.5Å

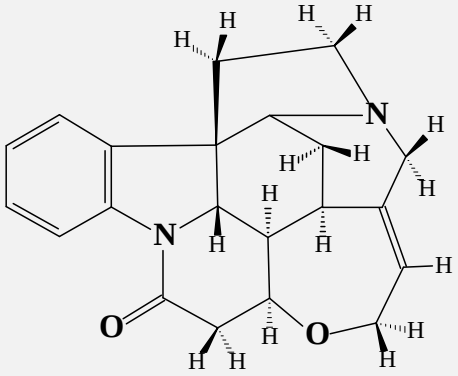
C(11)上的H – C(8)上的H
≈ 3.8Å

C(11)上的H – C(7)上的H
≈ 4.7Å



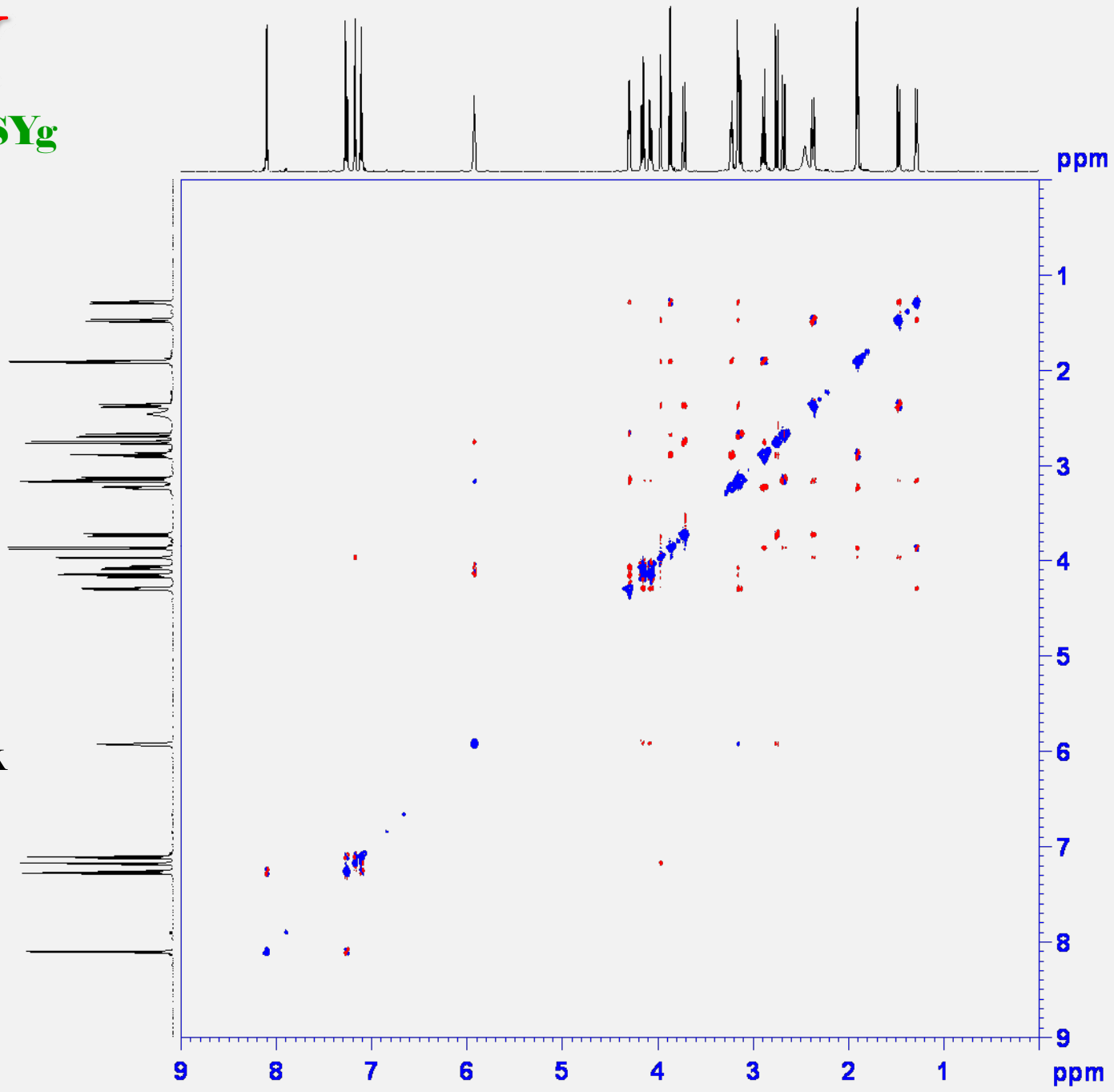
ROESY

IGRC_2D_ROESYg



Strychnine

color of the peak
positive peak
negative peak

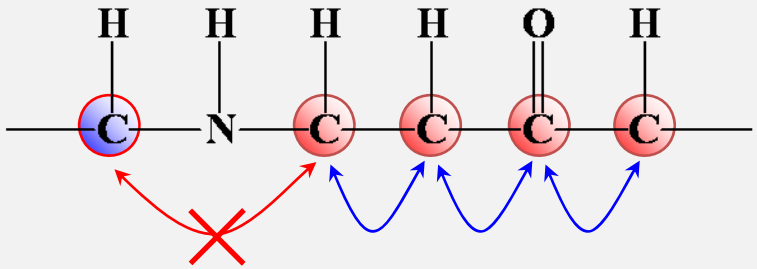


3.4 2D homonuclear ^{13}C

- **INADEQUATE**

  (Incredible Natural Abundance Double Quantum Transfer Experiment Spectroscopy)

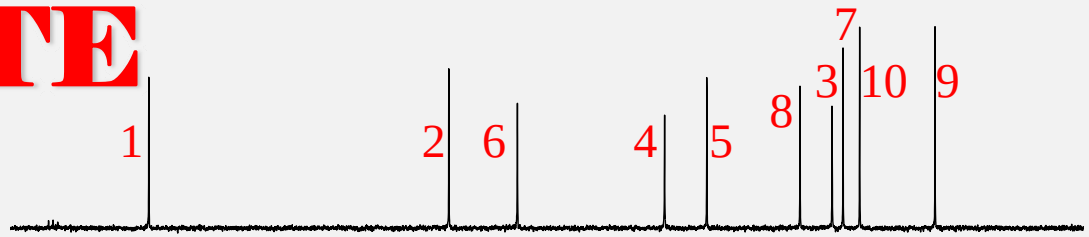
獲得$^{1}J_{C\text{-}C}$之關係

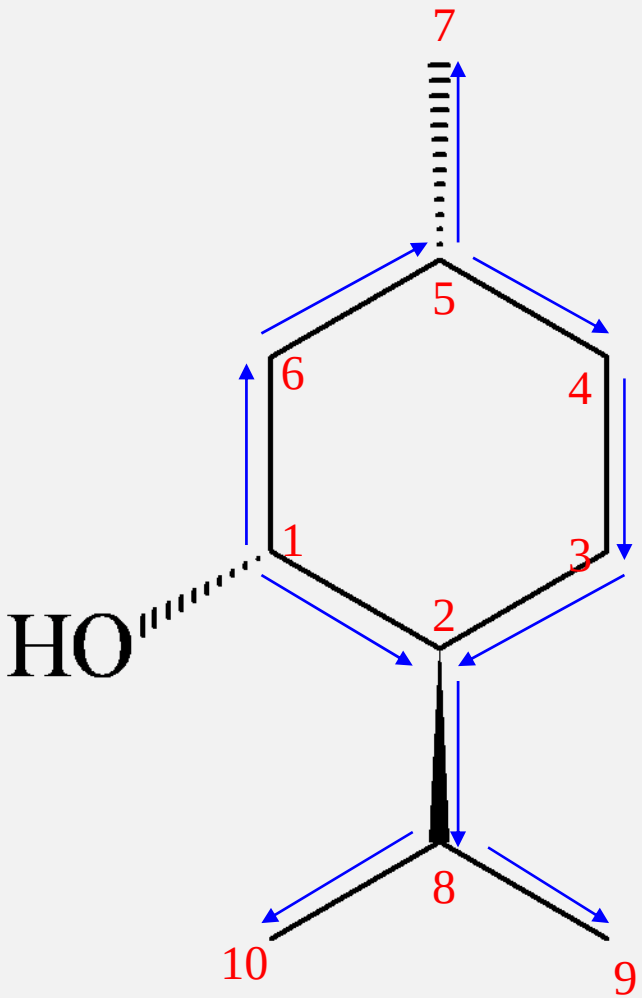


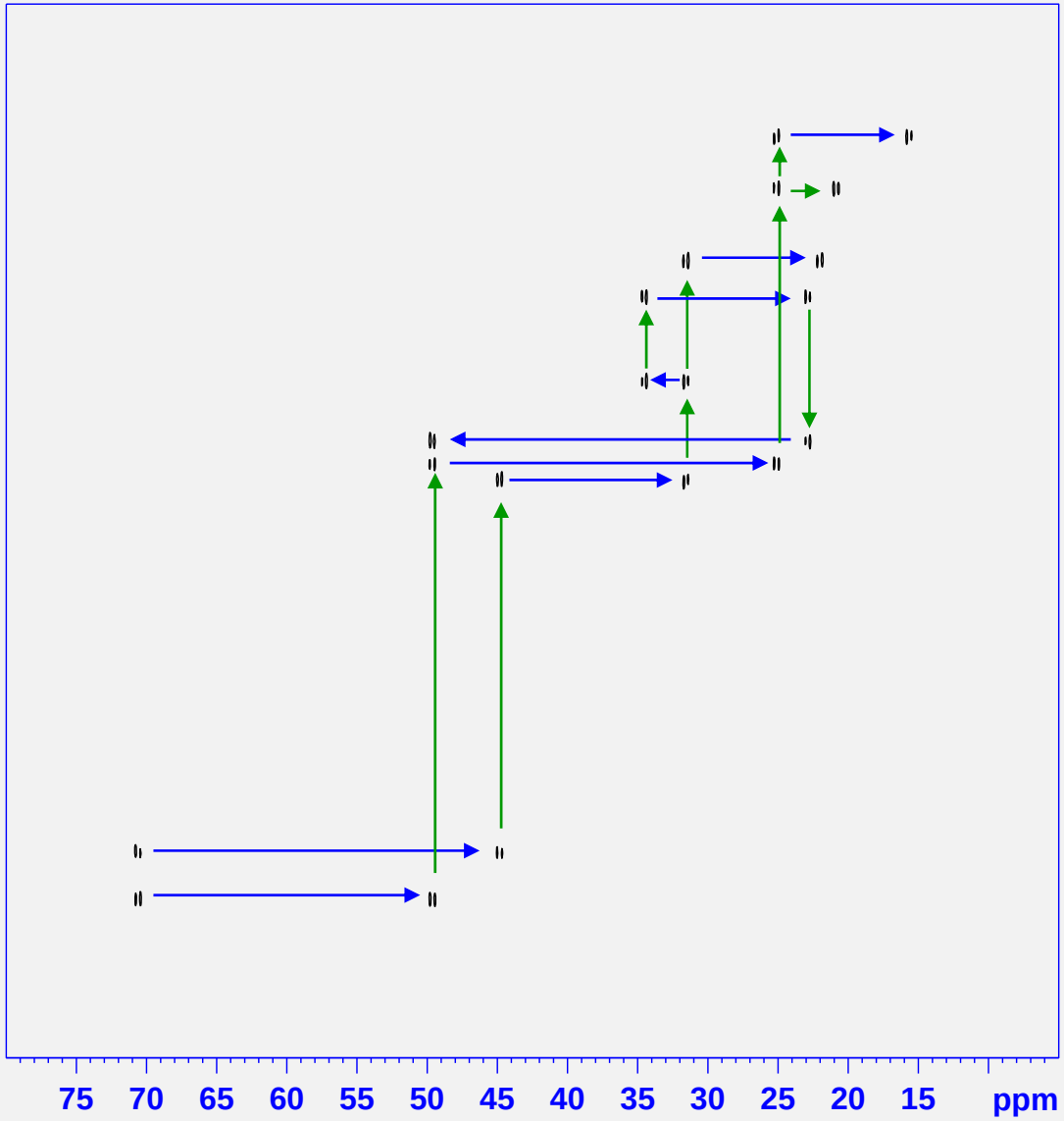
CryoProbe

INADEQUATE

IGRC_2D_INADEQUATE







4. 2D heteronuclear NMR

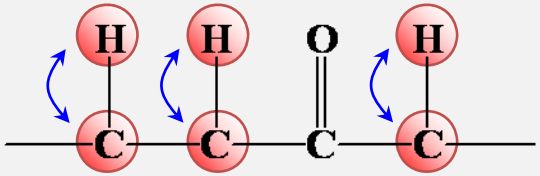
- **HMQC**
  - 1GRC_2D_HMQC
  - 1GRC_2D_HMQCs
- **HSQC**
  - 1GRC_2D_HSQC
  - 1GRC_2D_HSQC-echo
  - 1GRC_2D_HSQC-suppression
- **H2BC**
  - 1GRC_2D_H2BC
- **HMBC**
  - 1GRC_2D_HMBC
  - 1GRC_2D_HMBC-suppression
- **CIGAR-HMBC**
  - 1GRC_2D_HMBC-CIGAR

4.1 2D heteronuclear NMR

☼ HMQC

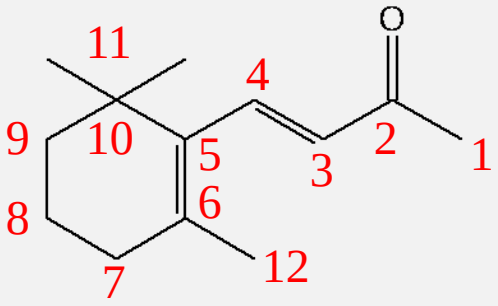
(Heteronuclear Multiple Quantum Correlation)

獲得$^{1}J_{H\text{-}X}$之關係

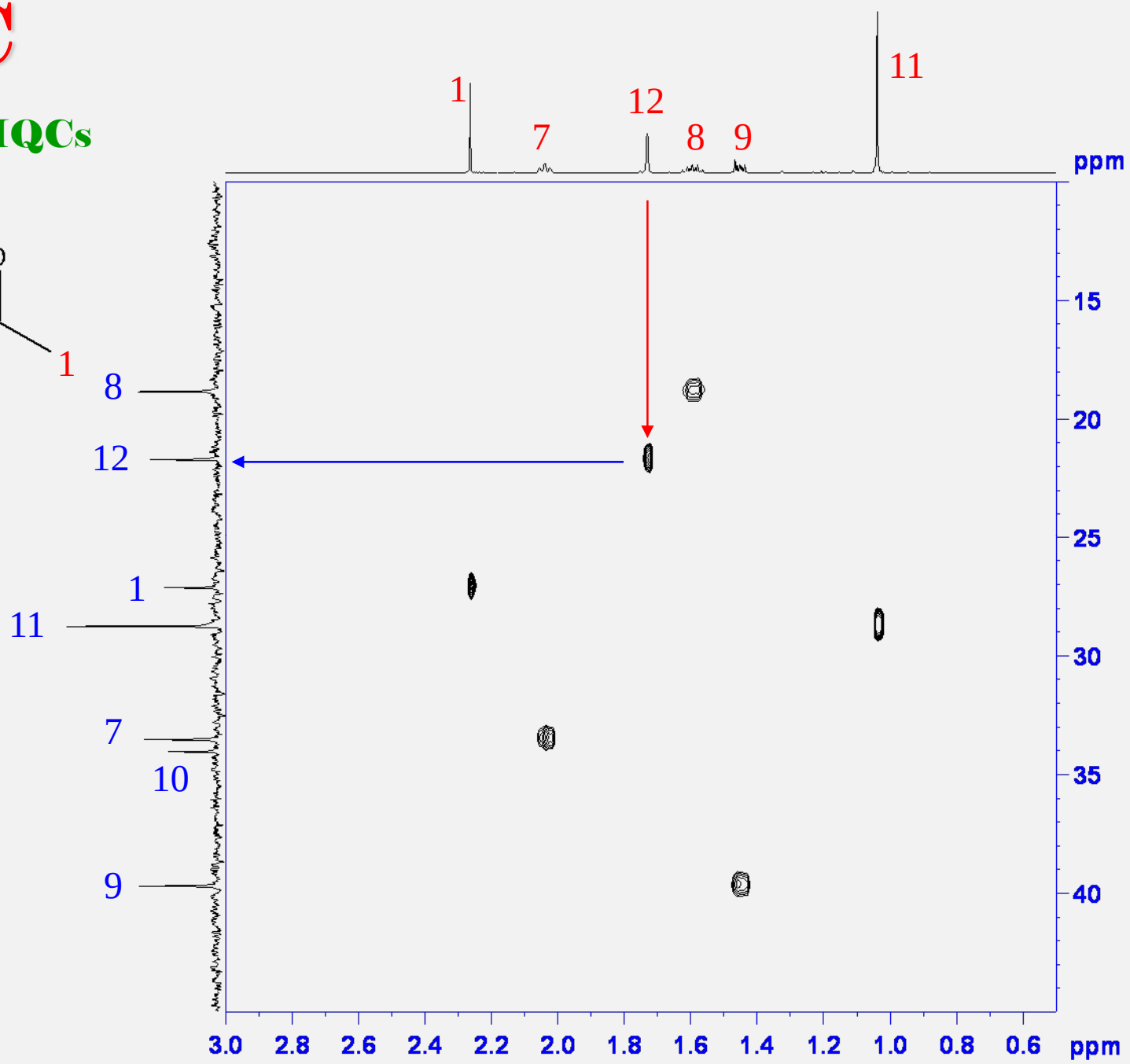


HMQC

IGRC_2D_HMQCs



β-ionone



4.2 2D heteronuclear NMR

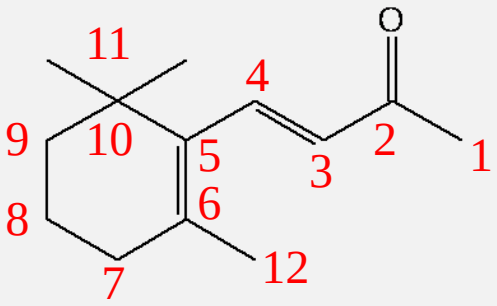
☀ HSQC

(Heteronuclear Single Quantum Correlation)

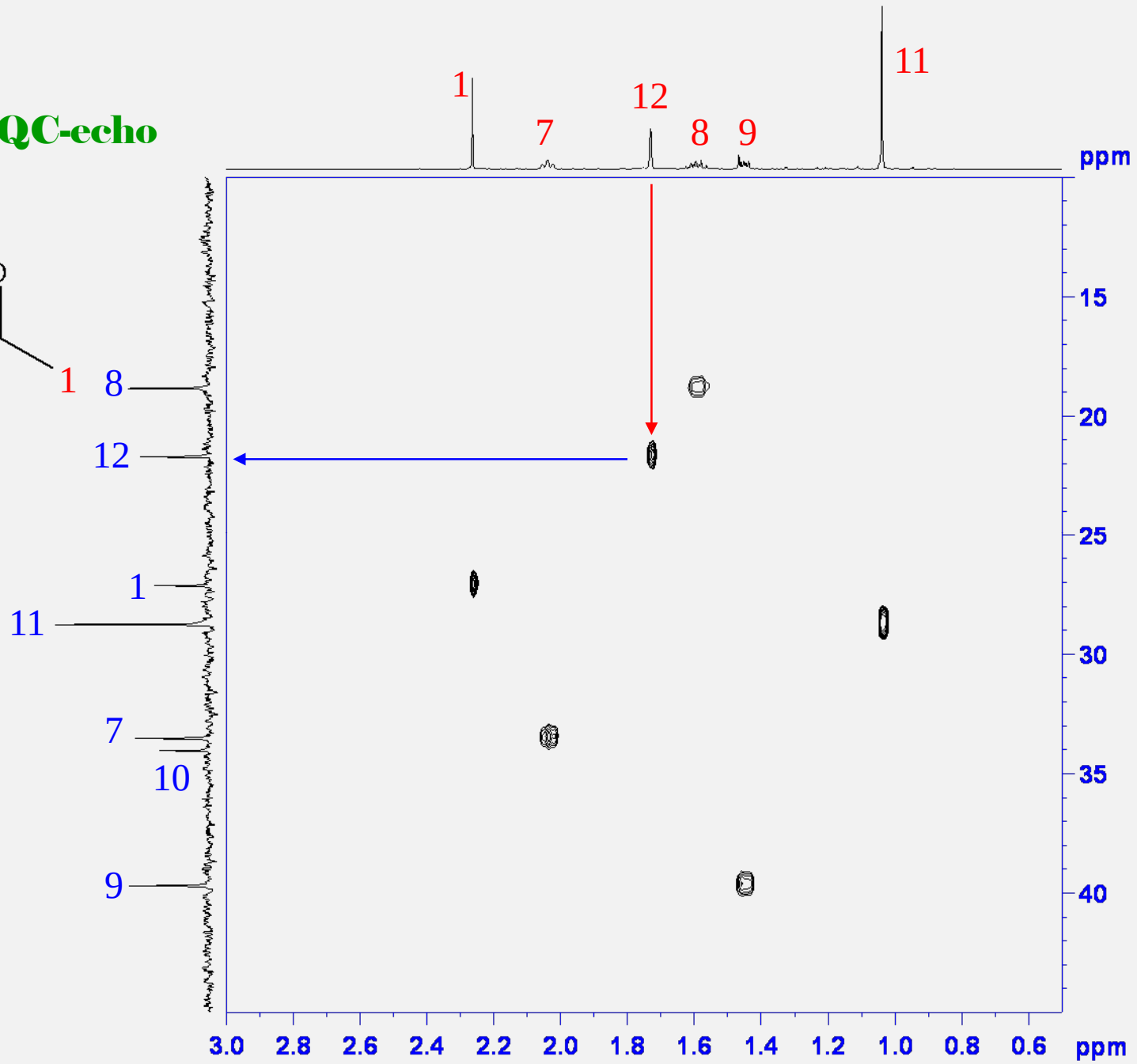
獲得$^{1}J_{H\text{-}X}$之關係

HSQC

IGRC_2D_HSQC-echo



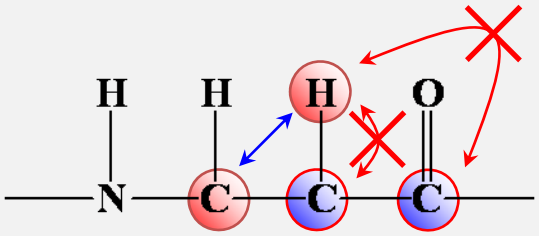
β-ionone



4.3 2D heteronuclear NMR

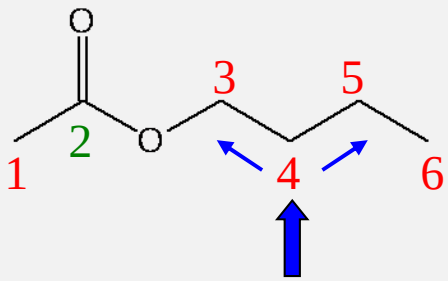
☼ H2BC

獲得$^{2}J_{H\text{-}XH}$之關係

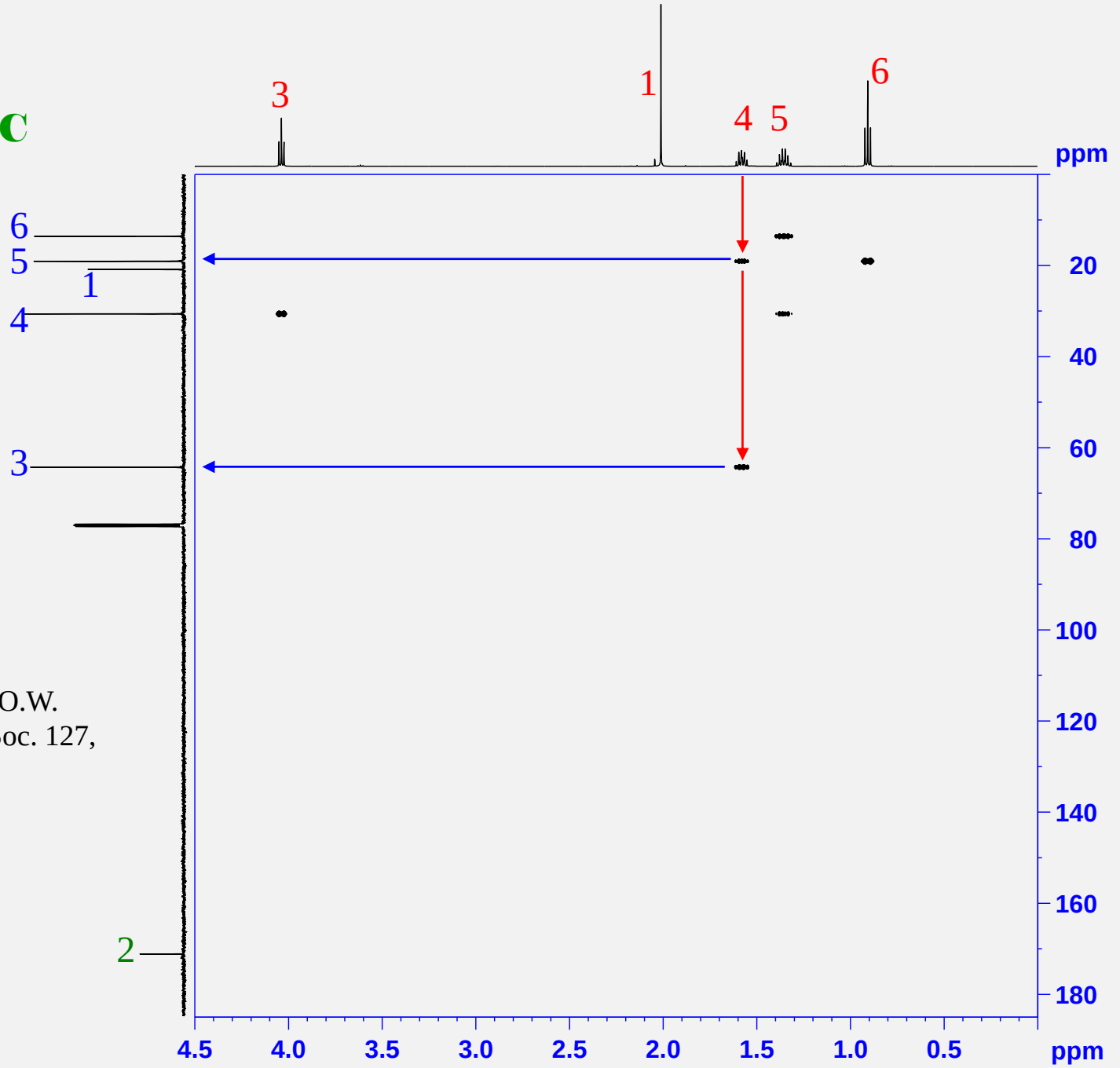


H2BC

IGRC_2D_H2BC



n-butyl acetate



N.T. Nyberg, J.O. Duus & O.W. Soerensen, J. Am. Chem. Soc. 127, 6154-6155 (2005)

4.4 2D heteronuclear NMR

☀ HMBC

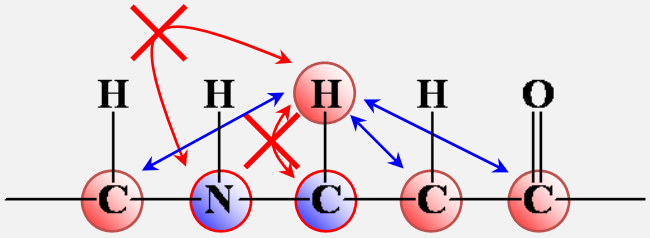
(Heteronuclear Multiple Bond Correlation)

獲得$^{n}J_{H\text{-}X}$之關係 **(n ≥ 2)**

☀ CIGAR-HMBC

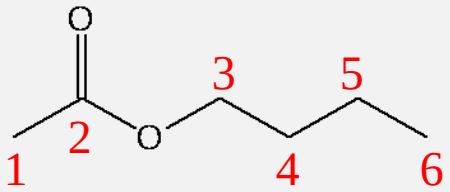
(Constant Time Inverse-detected Gradient Accordion Rescaled-HMBC)

獲得$^{n}J_{H\text{-}X}$之關係 **(n ≥ 2)**，可調整偵測長距離之**H-X**偶合常數

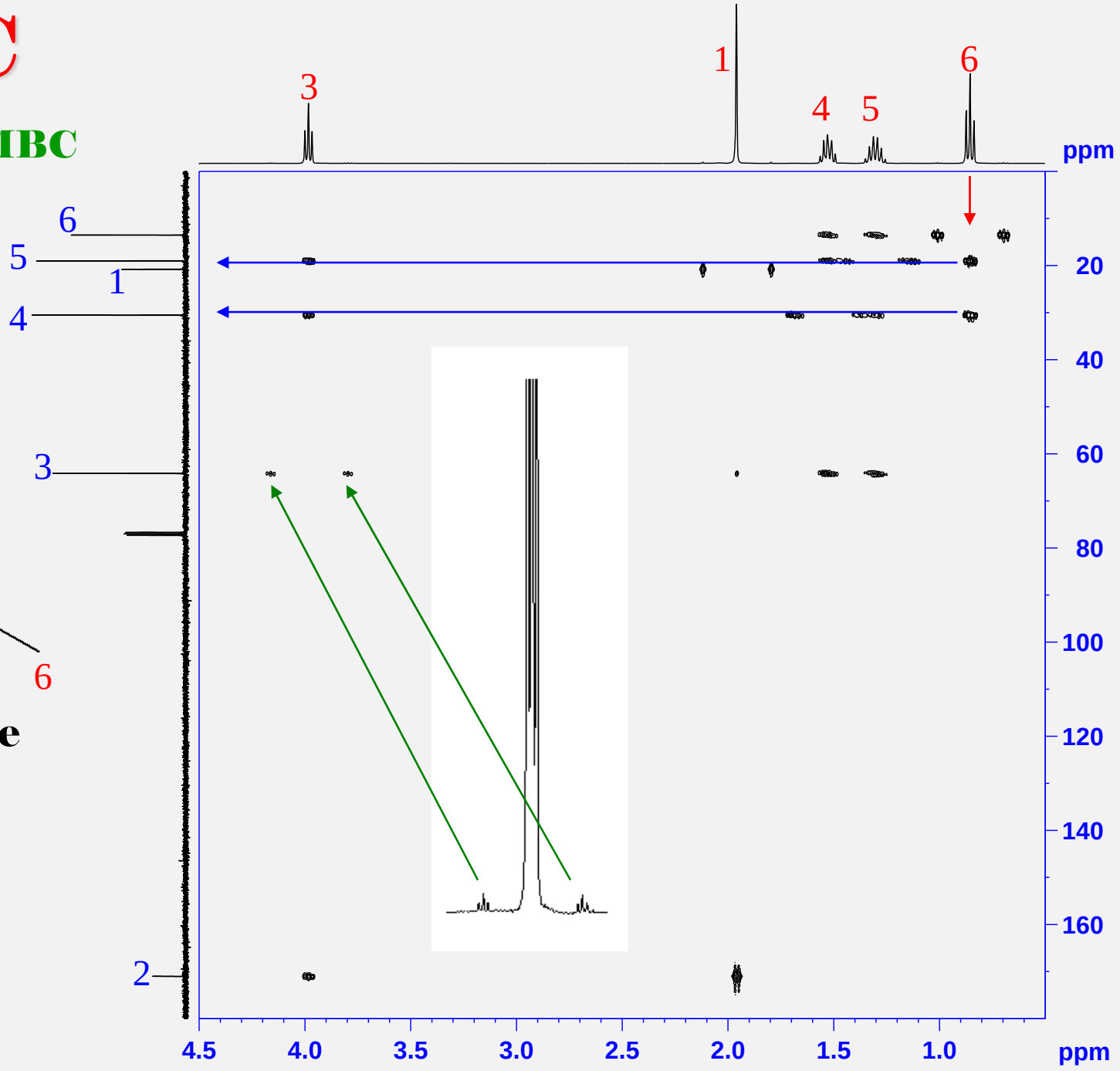


HMBC

IGRC_2D_HMBC

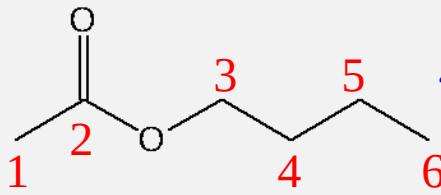


n-butyl acetate



CIGAR-HMBC

IGRC_2D_HMBC-CIGAR

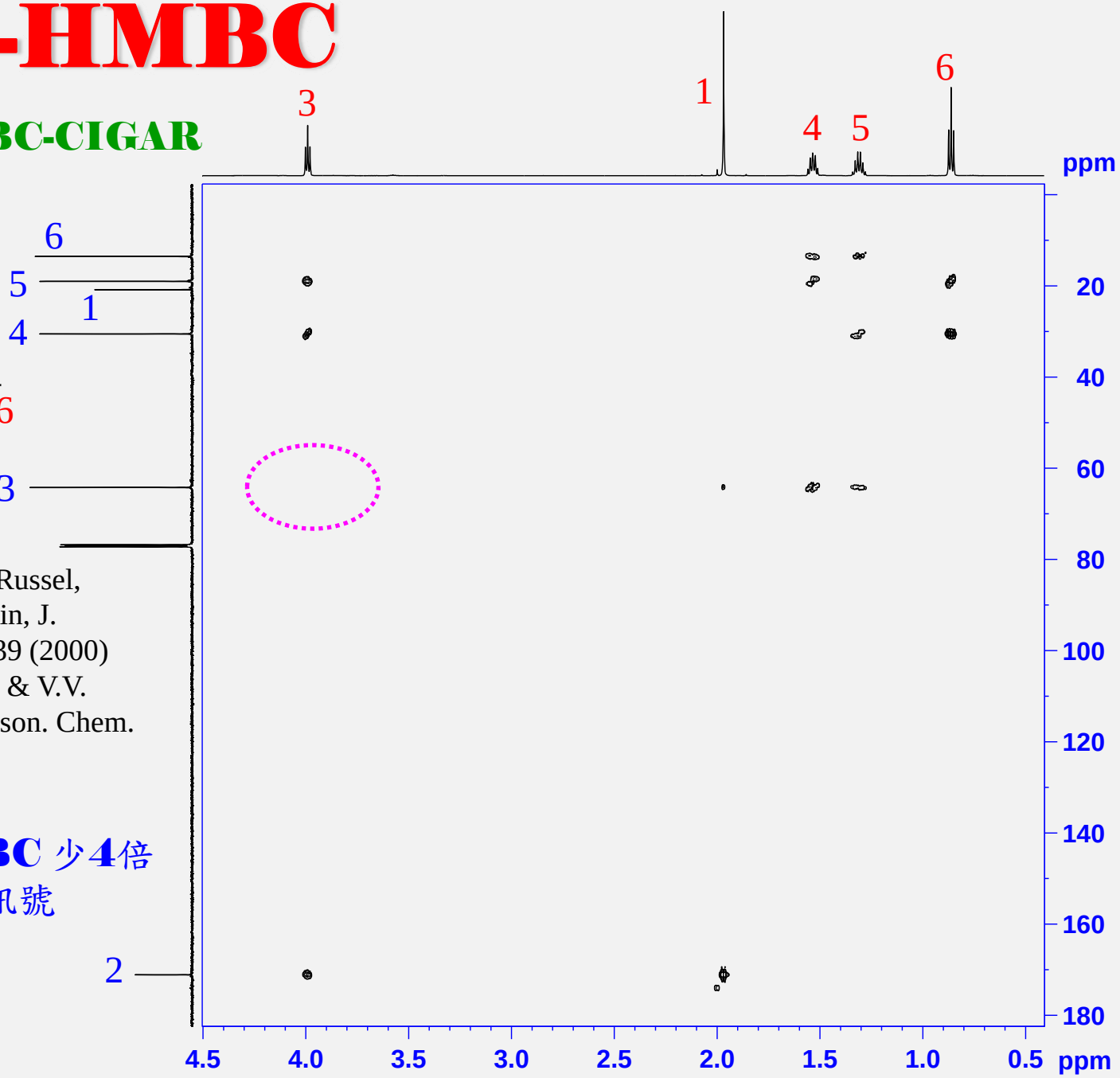


n-butyl acetate

1. V.V. Krishnamurthy, D.J. Russel, C.E. Hadden & G.E. Martin, J. Magn. Reson. 146, 232-239 (2000)
2. C.E. Hadden, G.E. Martin & V.V. Krishnamurthy, Magn. Reson. Chem. 38, 143-147 (2000)

1. 訊號強度較**HMBC** 少**4**倍

2. 可去除**satellite** 訊號



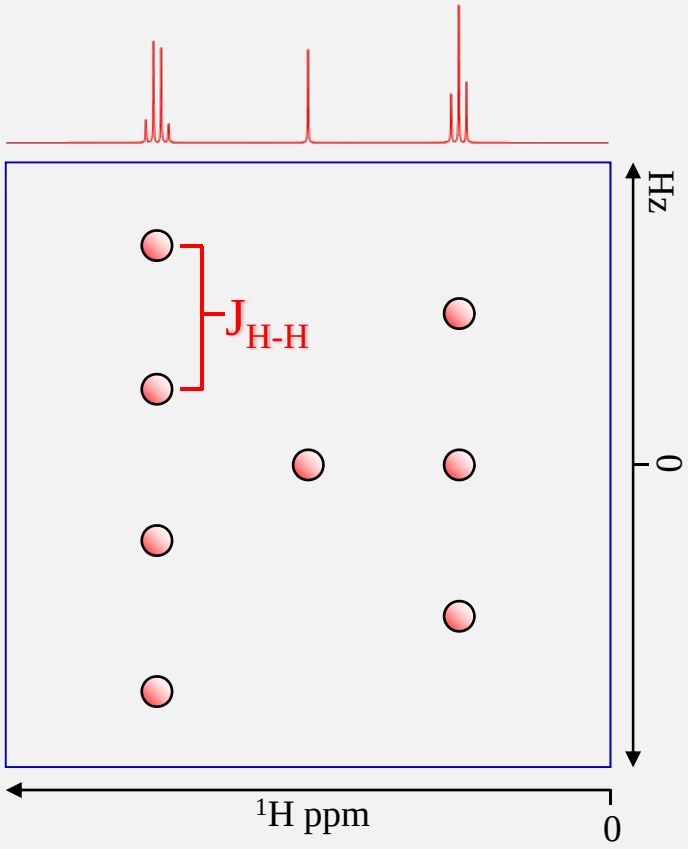
5. Measurement of coupling constants

- **Homonuclear J-resolved**
  - 1GRC_2D_Jres
- **Heteronuclear J-resolved**
  - 1GRC_2D_HJres
  - 1GRC_2D_sel.HJres
  - 1GRC_2D_no decouple HSQC
  - 1GRC_2D_HETLOC
  - 1GRC_2D_HSQC-HECADE
  - 1GRC_2D_ps-HMBC
  - 1GRC_2D_G-BIRD-HSQMBC

5.1 Measurement of coupling constants

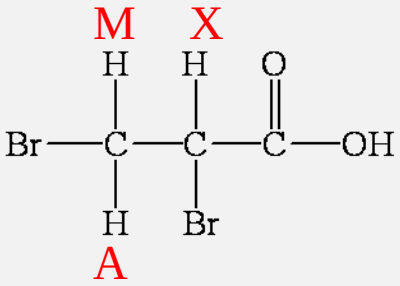
☀ homonuclear J-resolved

獲得$J_{H\text{-}H}$之偶合常數

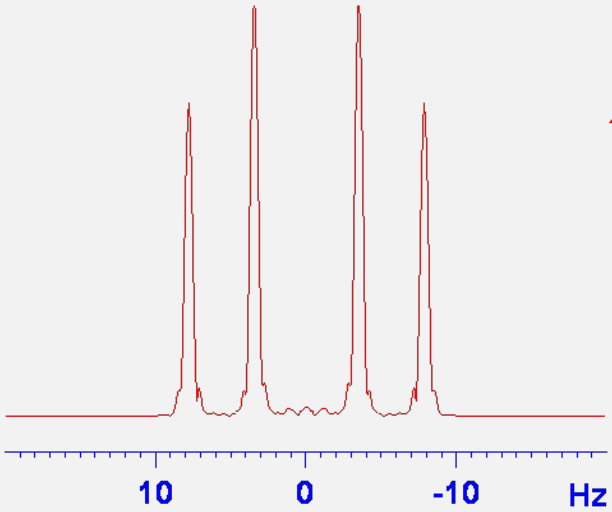


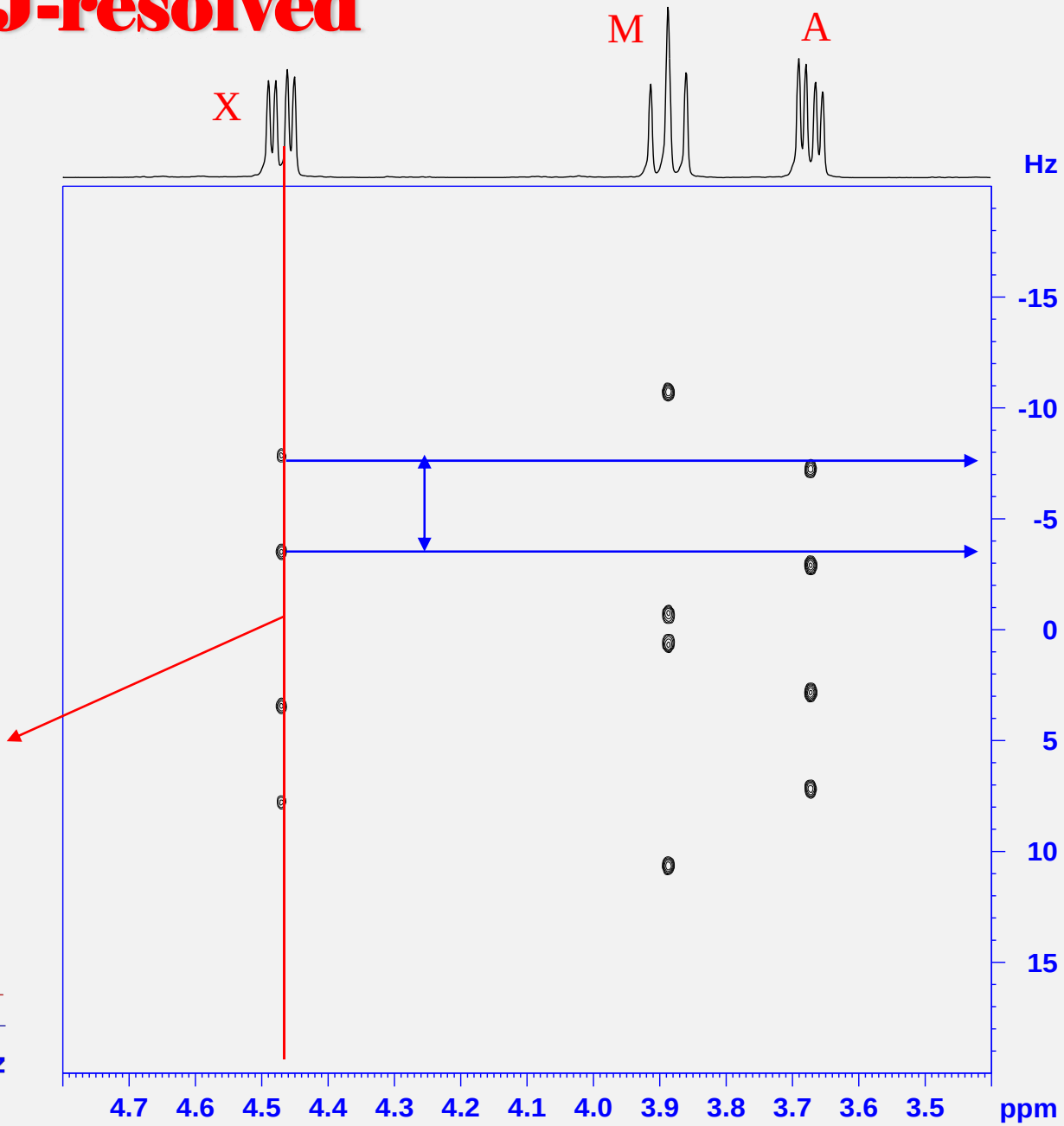
homonuclear J-resolved

IGRC_2D_Jres



2,3-dibromo-propionic acid

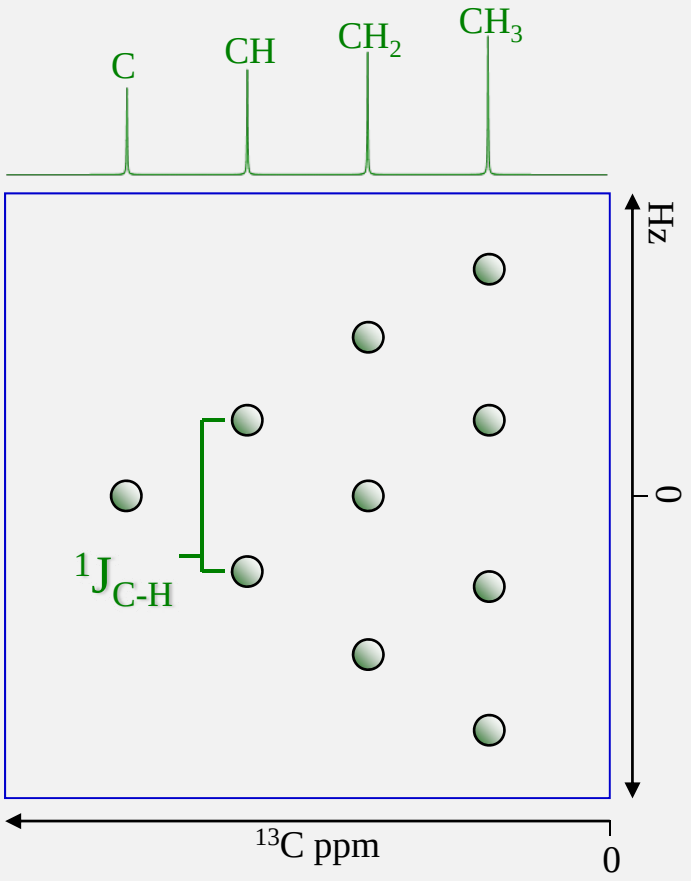




5.2 Measurement of coupling constants

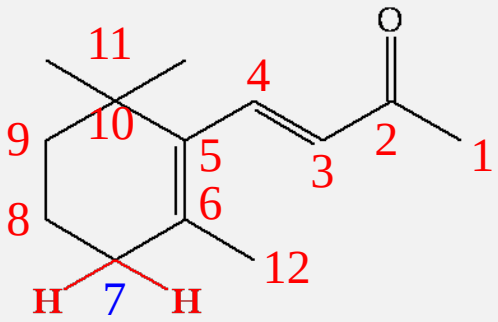
heteronuclear J-resolved

獲得J_{C-H}之偶合常數

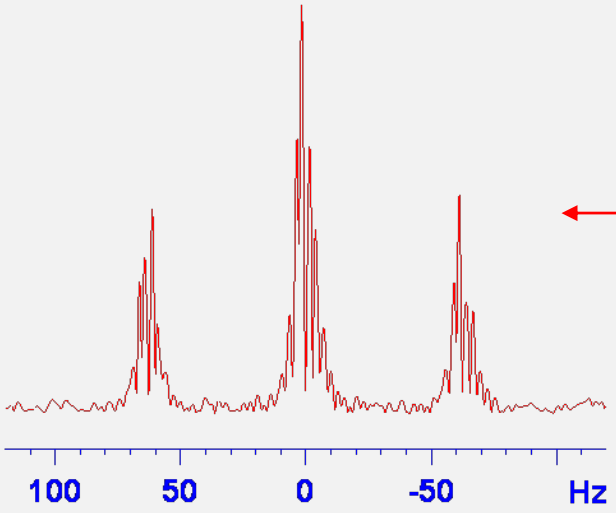


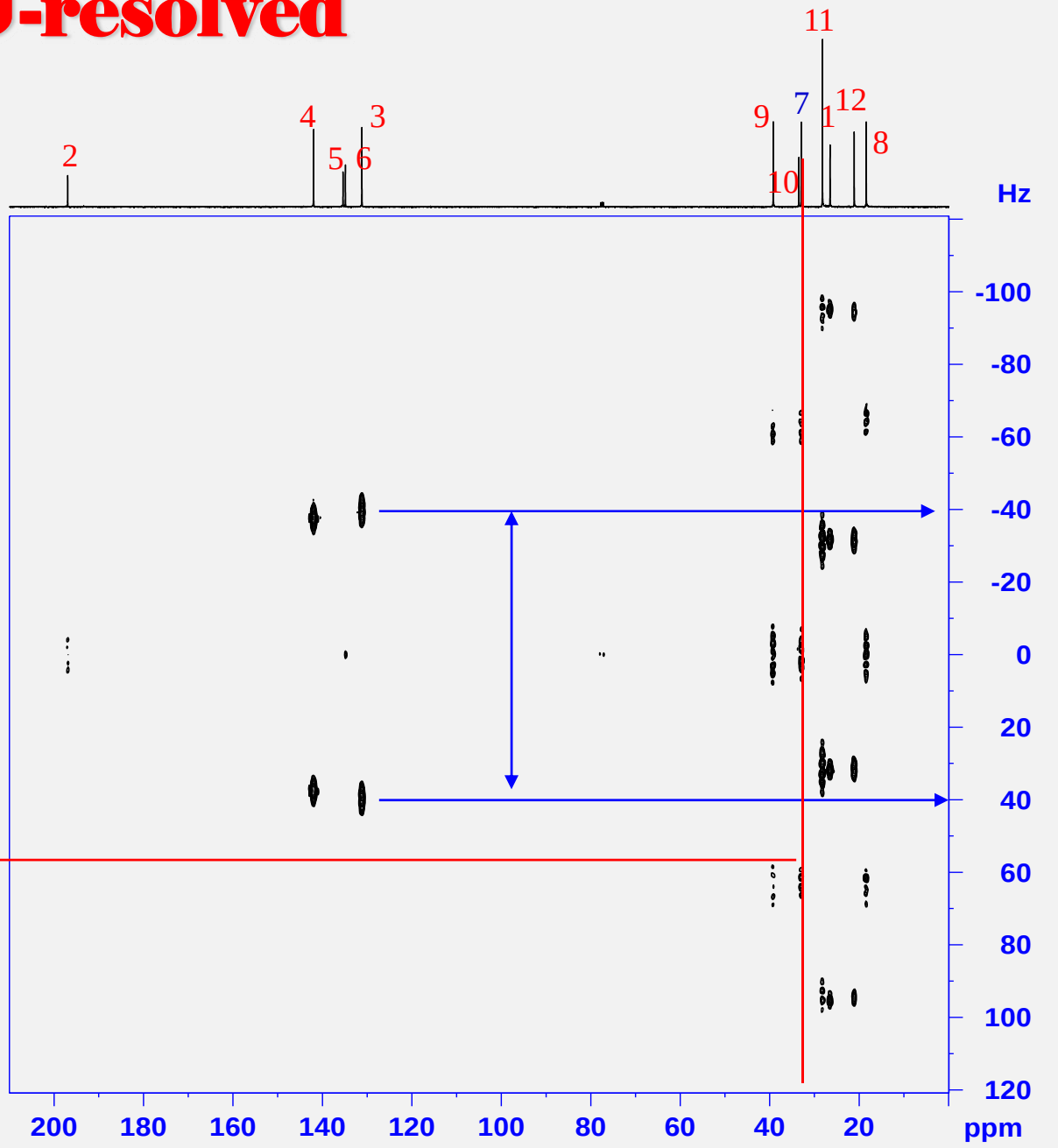
heteronuclear J-resolved

IGRC_2D_HJres



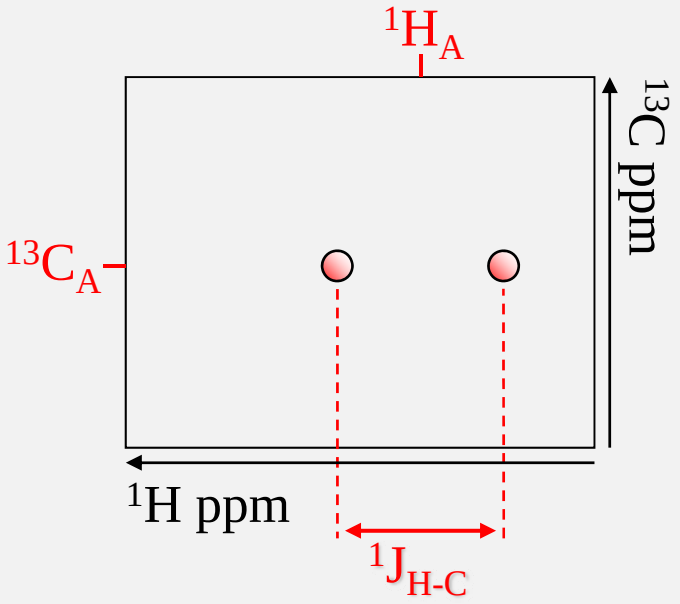
β-ionone

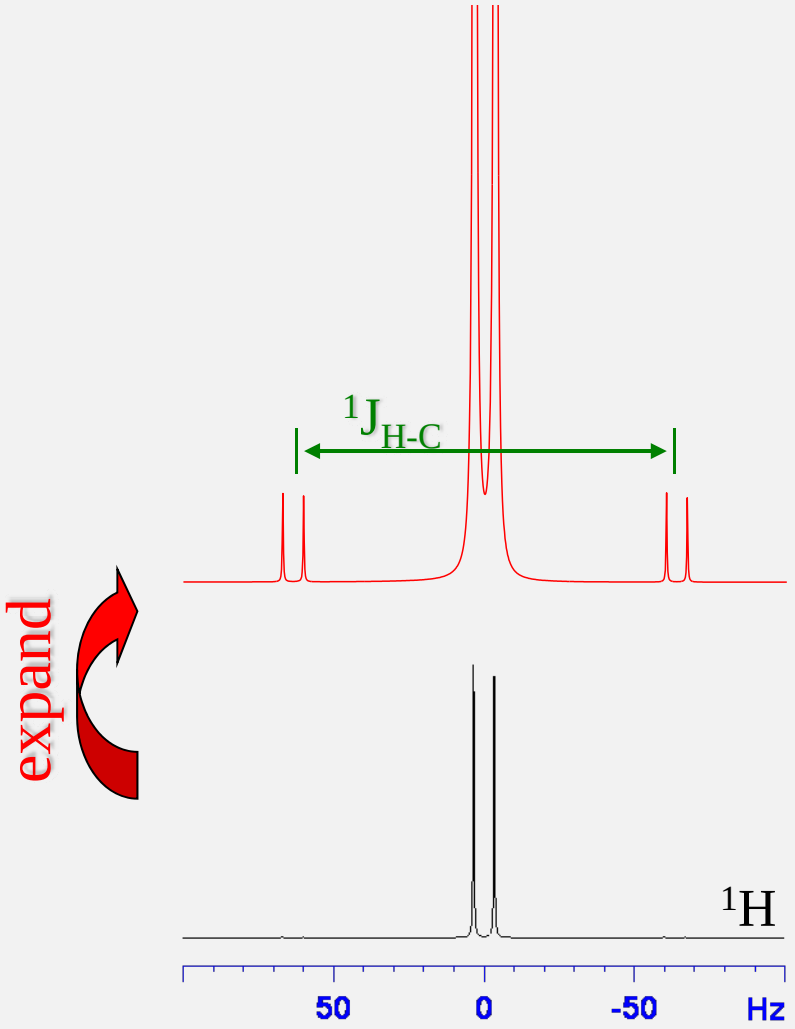




5.3 Measurement of coupling constants

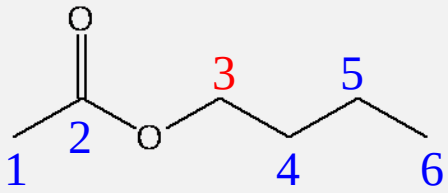
- **no decoupling HSQC**
  獲得$^{1}J_{H\text{-}HC}$之偶合常數



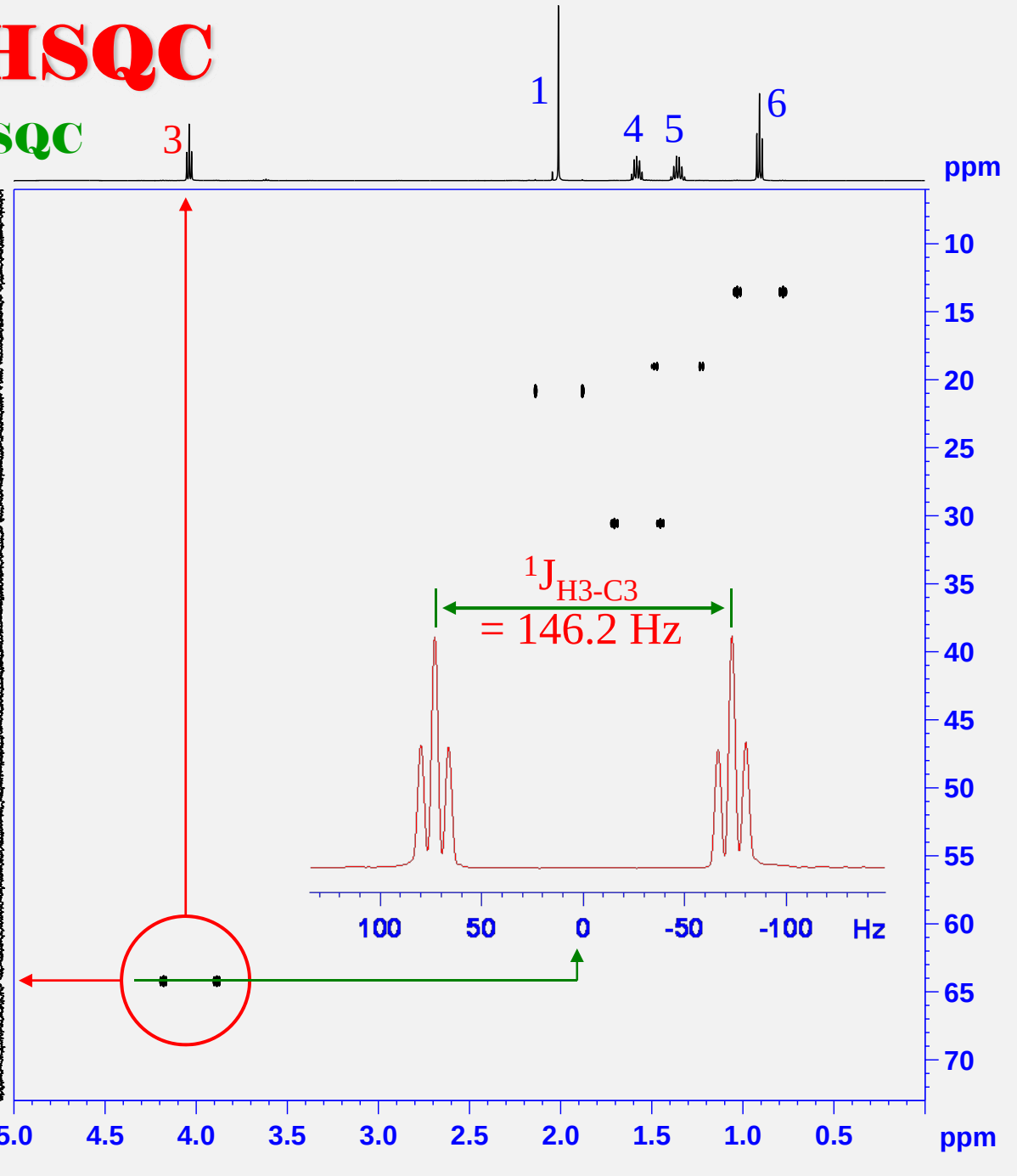


no decoupling HSQC

IGRC_2D_no decouple HSQC



n-butyl acetate

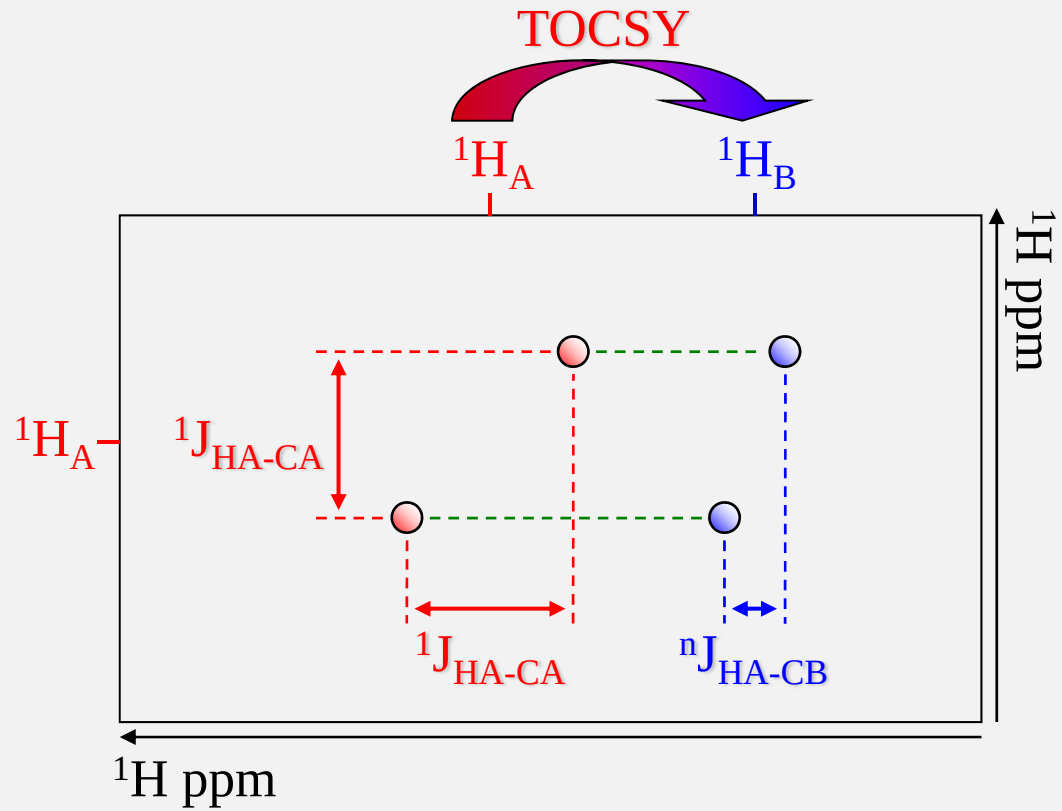


5.4 Measurement of coupling constants

☀ HETLOC

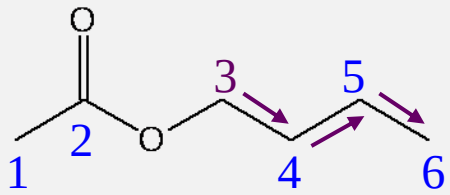
(HETeronuclear LOng-range Couplings)

獲得$^{n}J_{H\text{-}HC}$之偶合常數(n ≥ 1)

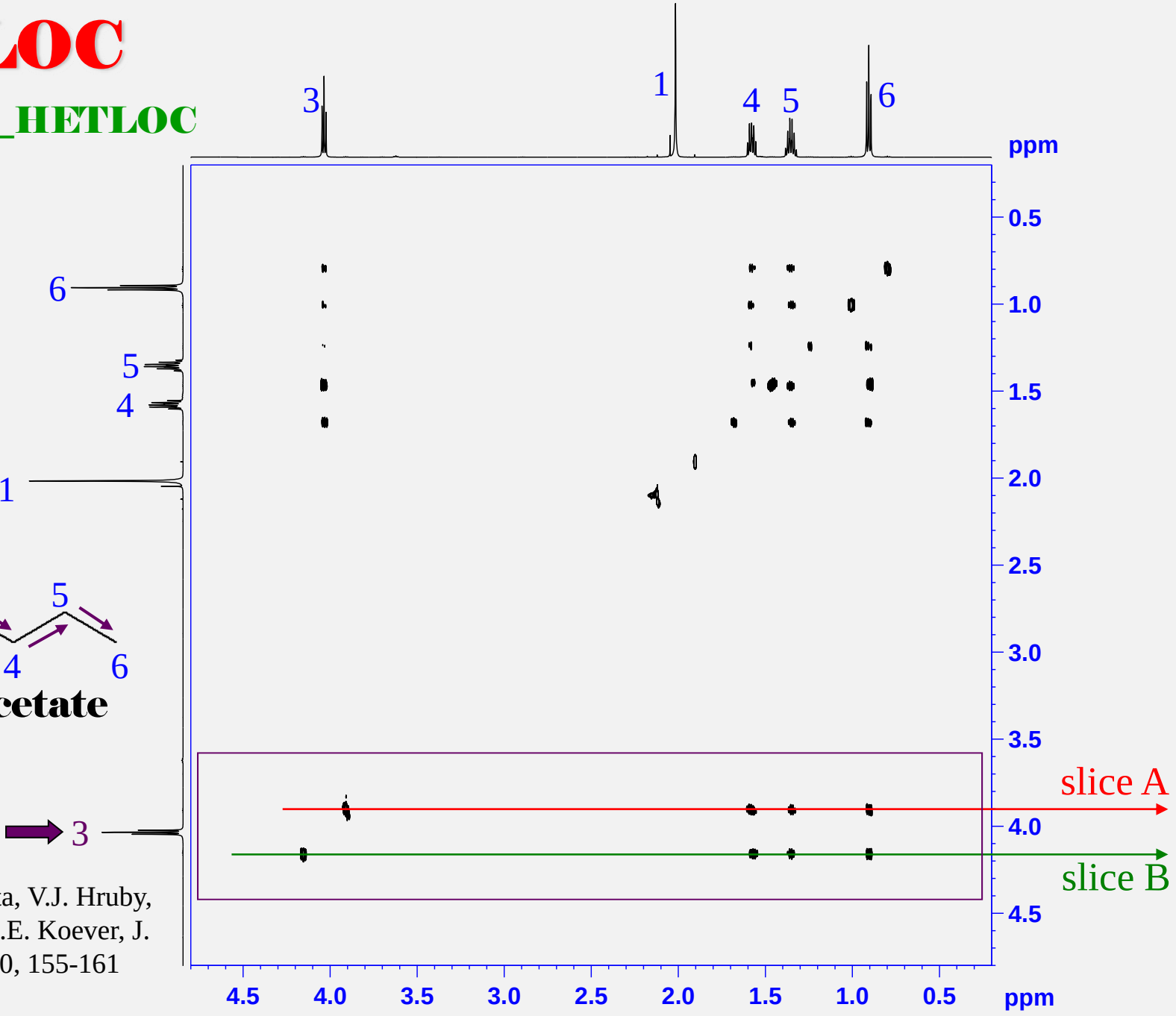


HETLOC

IGRC_2D_HETLOC



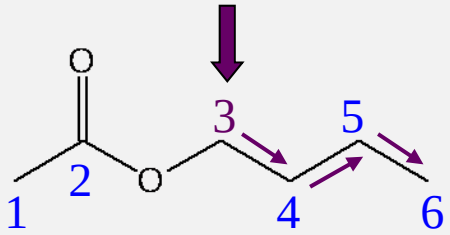
n-butyl acetate



1. D. Uhrin, G. Batta, V.J. Hruby, P.N. Barlow & K.E. Koever, J. Magn. Reson. 130, 155-161 (1998)

HETLOC

IGRC_2D_HETLOC



n-butyl acetate

H3 → H4 → H5 → H6

$^{1}J_{H3-C3}$ = 146.2 Hz

$^{2}J_{H3-C4}$ = 4.4 Hz

$^{3}J_{H3-C5}$ = 4.1 Hz

$^{4}J_{H3-C6}$ ≈ 0 Hz

slice A

→ H3

slice B

4.2 4.1 4.0 ppm

1.60 ppm

1.35 ppm

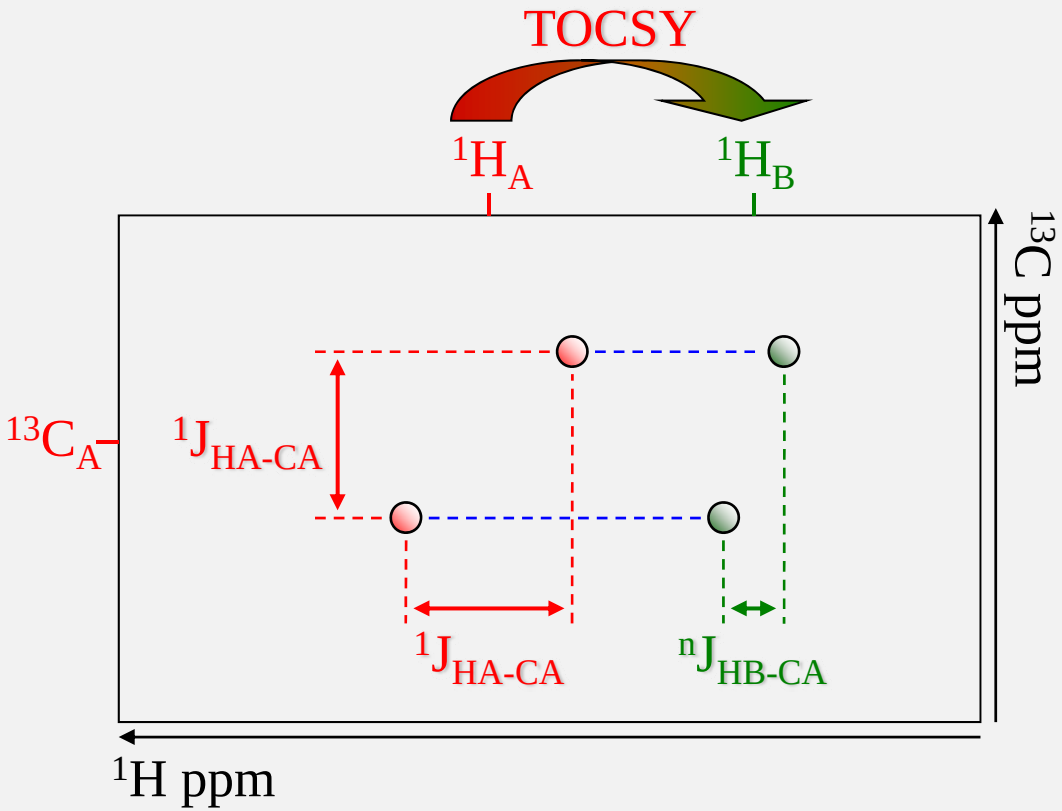
0.90 ppm

5.5 Measurement of coupling constants

☀ HSQC-HECADE

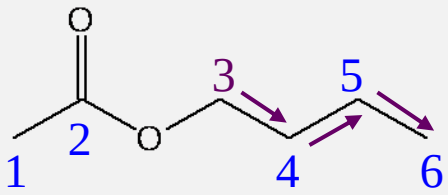
(HSQC-HEteronuclear Couplings from ASSCI-Domain Experiments with E.COSY-Type Cross Peaks)

獲得$^{n}J_{H\text{-}HC}$之偶合常數(n ≥ 1)



HSQC-HECADE

IGRC_2D_HSQC-HECADE

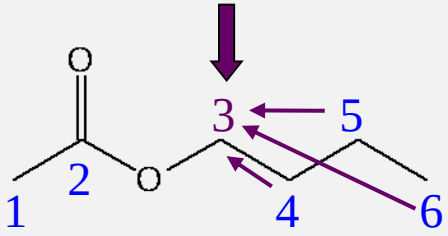


n-butyl acetate

W. Kozminski & D. Nanz, J. Magn. Reson. 142, 294-299 (2000)

HSQC-HECADE

IGRC_2D_HSQC-HECADE



n-butyl acetate

H3 → H4 → H5 → H6

$^{1}J_{H3-C3}$ = 146.2 Hz

$^{2}J_{H4-C3}$ = 4.6 Hz

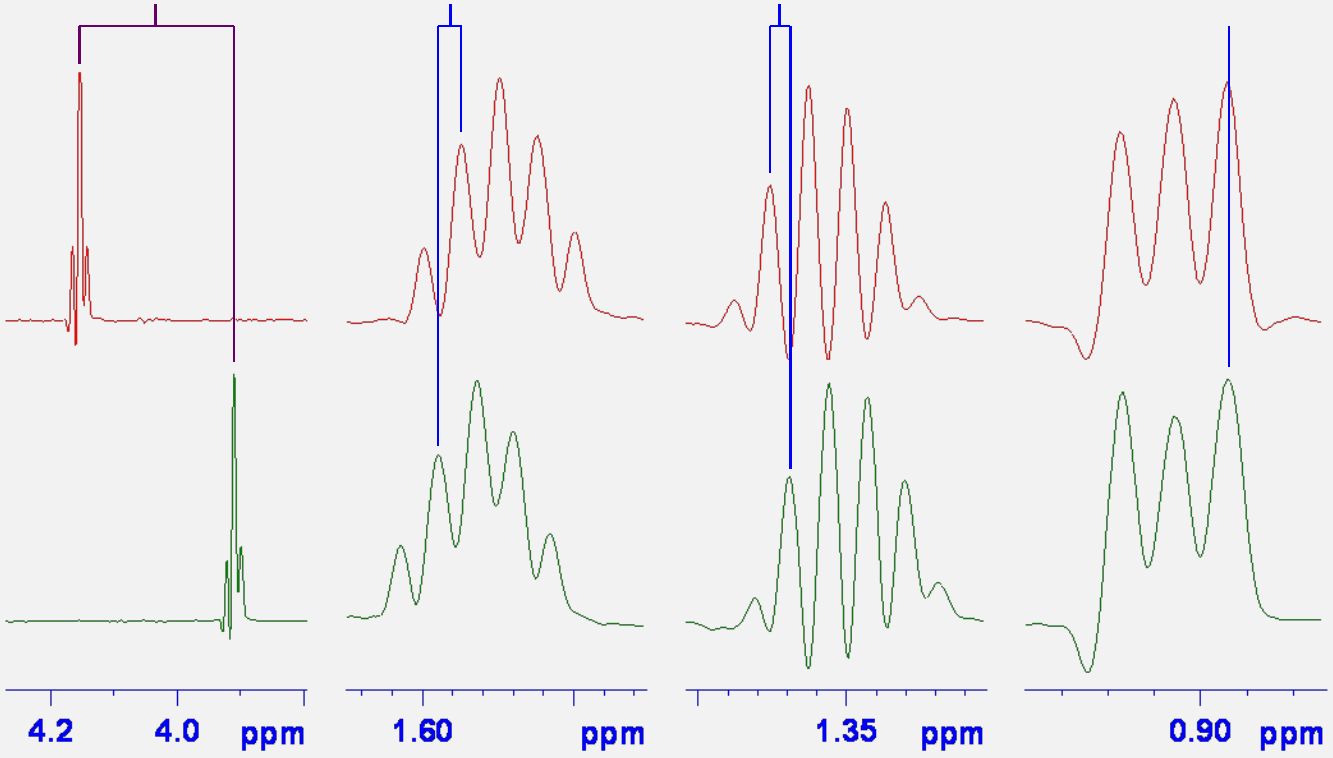
$^{3}J_{H5-C3}$ = 4.1 Hz

$^{4}J_{H6-C3}$ ≈ 0 Hz

slice A →

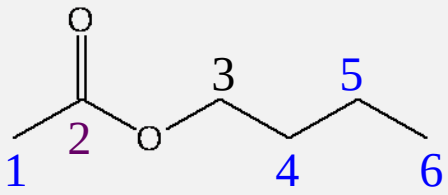
→ C3

slice B →



5.6 ps-HMBC

IGRC_2D_ps-HMBC



n-butyl acetate

D.O. Cicero, G. Barbato & R. Bazzo, J. Magn. Reson. 148, 209-213 (2001)

color of the peak
positive peak
negative peak

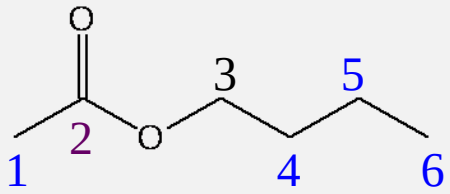
$^{2}J_{H4-C3}$ $^{3}J_{H5-C3}$

$^{3}J_{H3-C2}$ $^{2}J_{H1-C2}$

ppm

ps-HMBC

1GRC_2D_ps-HMBC



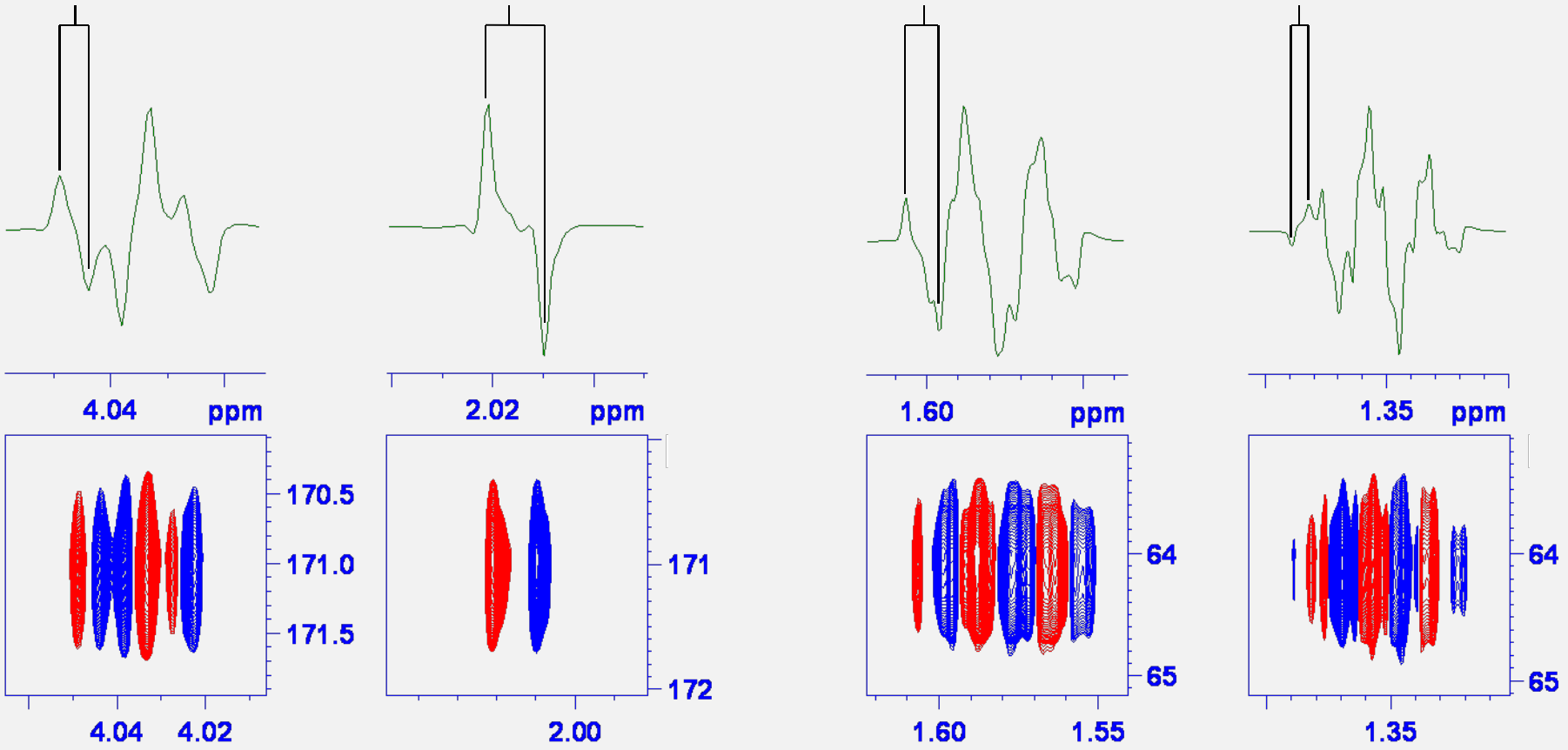
n-butyl acetate

$^{3}J_{H3\text{-}C2}$ = 3.1 Hz

$^{2}J_{H1\text{-}C2}$ = 6.7 Hz

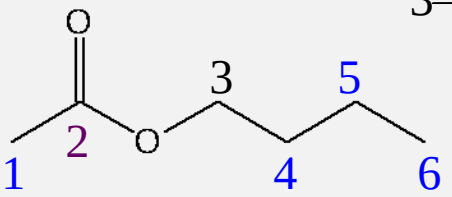
$^{2}J_{H4\text{-}C3}$ = 4.6 Hz

$^{3}J_{H5\text{-}C3}$ = 4.2 Hz

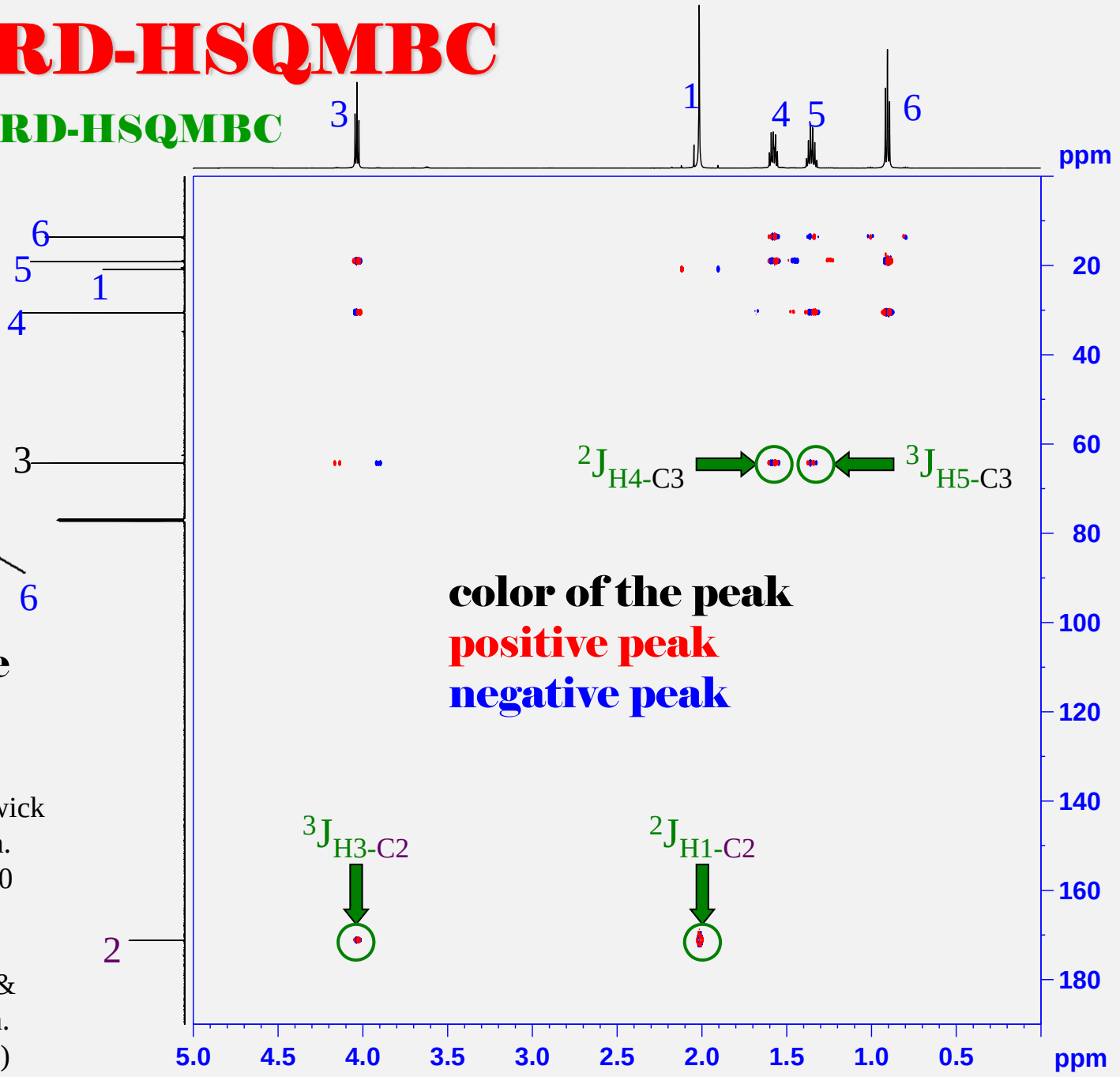


5.7 G-BIRD-HSQMBC

IGRC_2D_G-BIRD-HSQMBC



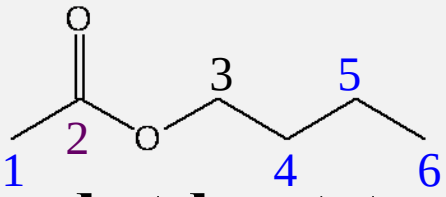
n-butyl acetate



1. B.L. Marquez, W.H. Gerwick & R.T. Williamson, Magn. Reson. Chem. 39, 499-530 (2001)
2. R.T. Williamson, B.L. Marquez, W.H. Gerwick & K.E. Kover, Magn. Reson. Chem. 38, 265-273 (2000)

G-BIRD-HSQMBC

IGRC_2D_G-BIRD-HSQMBC



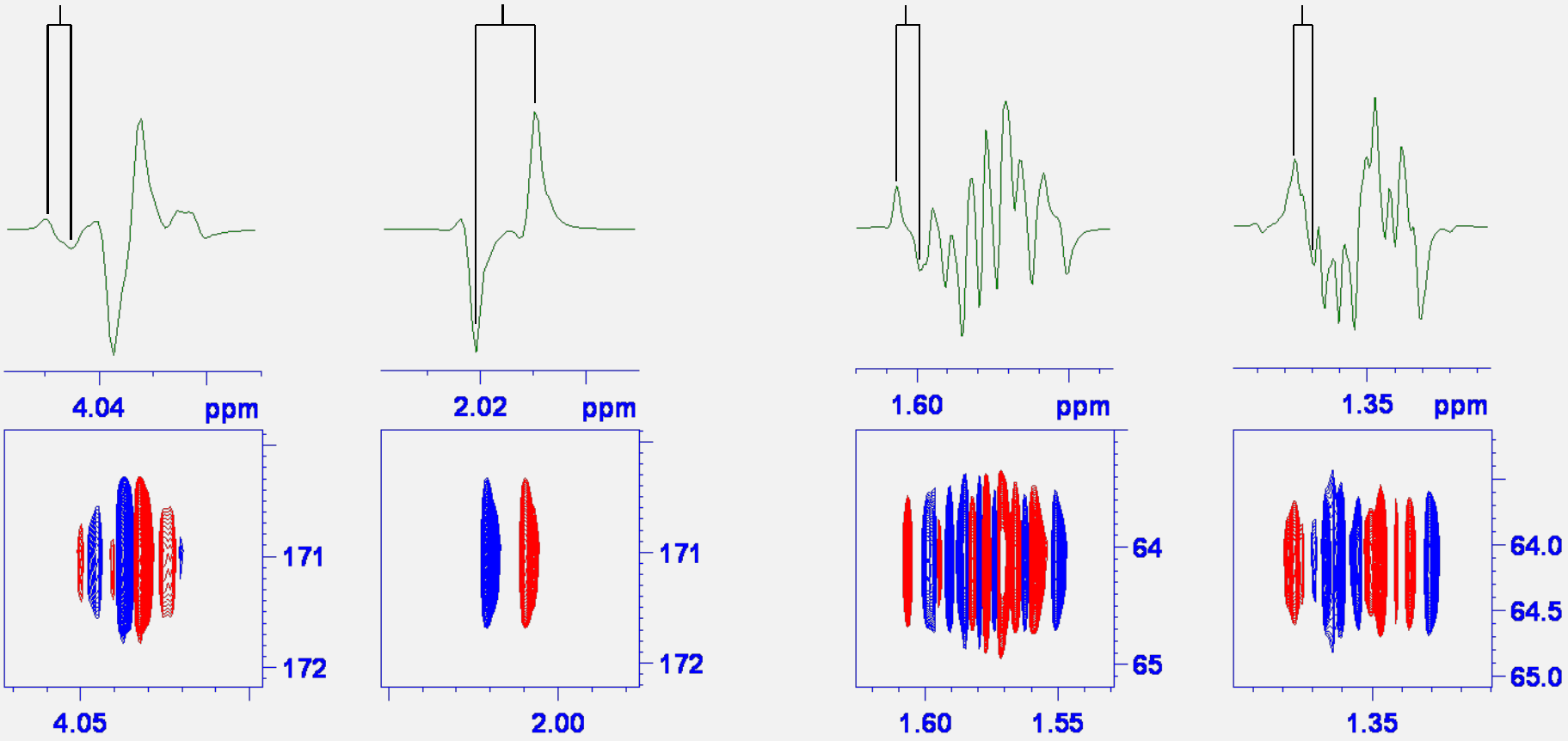
n-butyl acetate

$^{3}J_{H3-C2}$ = 2.9 Hz

$^{2}J_{H1-C2}$ = 6.6 Hz

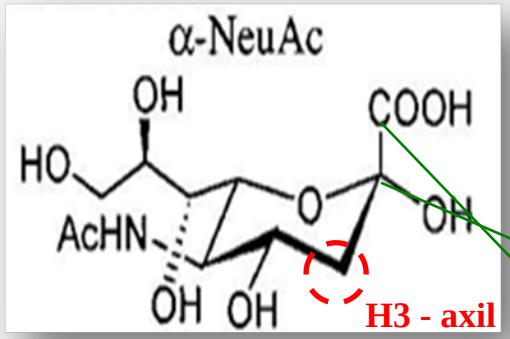
$^{2}J_{H4-C3}$ = 4.5 Hz

$^{3}J_{H5-C3}$ = 4.1 Hz



5.8 Sel_heteronuclear J-resolved

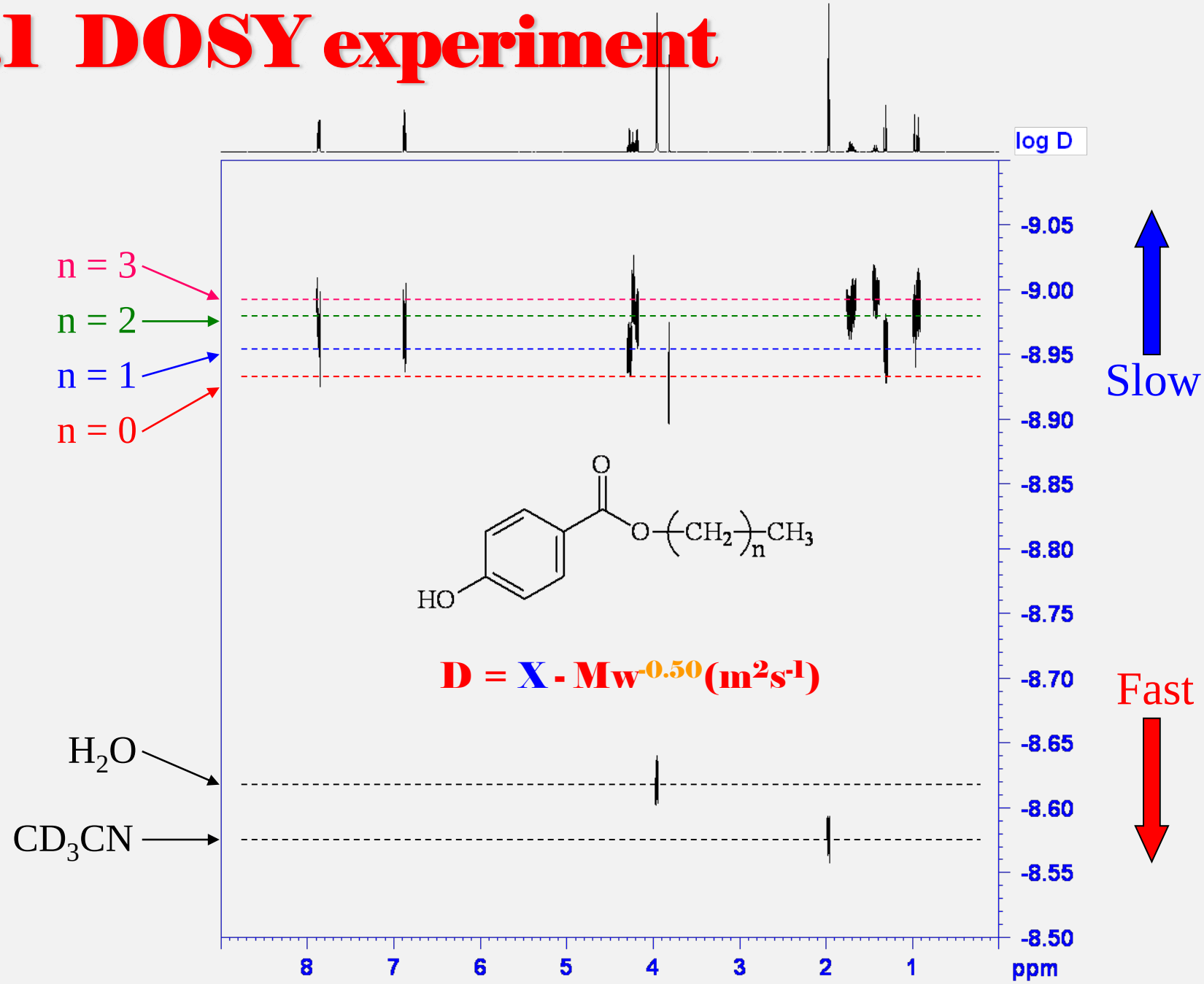
1GRC_2D_sel.HJres



6. DOSY (Diffusion-Ordered SpectrocopY)
(擴散排序光譜)

- **DOSY experiment:** 區分混合物中物種數目
- 利用混合物中各成分之擴散係數不同(溶液中的移動速度)進行區分
- 擴散係數與分子之大小、形狀、環境溫度與溶劑之黏度有關
- 藉由擴散實驗區分在光譜中各訊號是否分屬於不同的分子

6.1 DOSY experiment



7. Other useful experiments

- **Selective - Selective Excitation**

  sel_COSY – NOESY
  sel_TOCSY – TOCSY
  sel_TOCSY – NOESY
  sel_TOCSY – ROESY
  sel_NOESY – TOCSY
  sel_ROESY – TOCSY
  sel_NOESY – NOESY

7.1

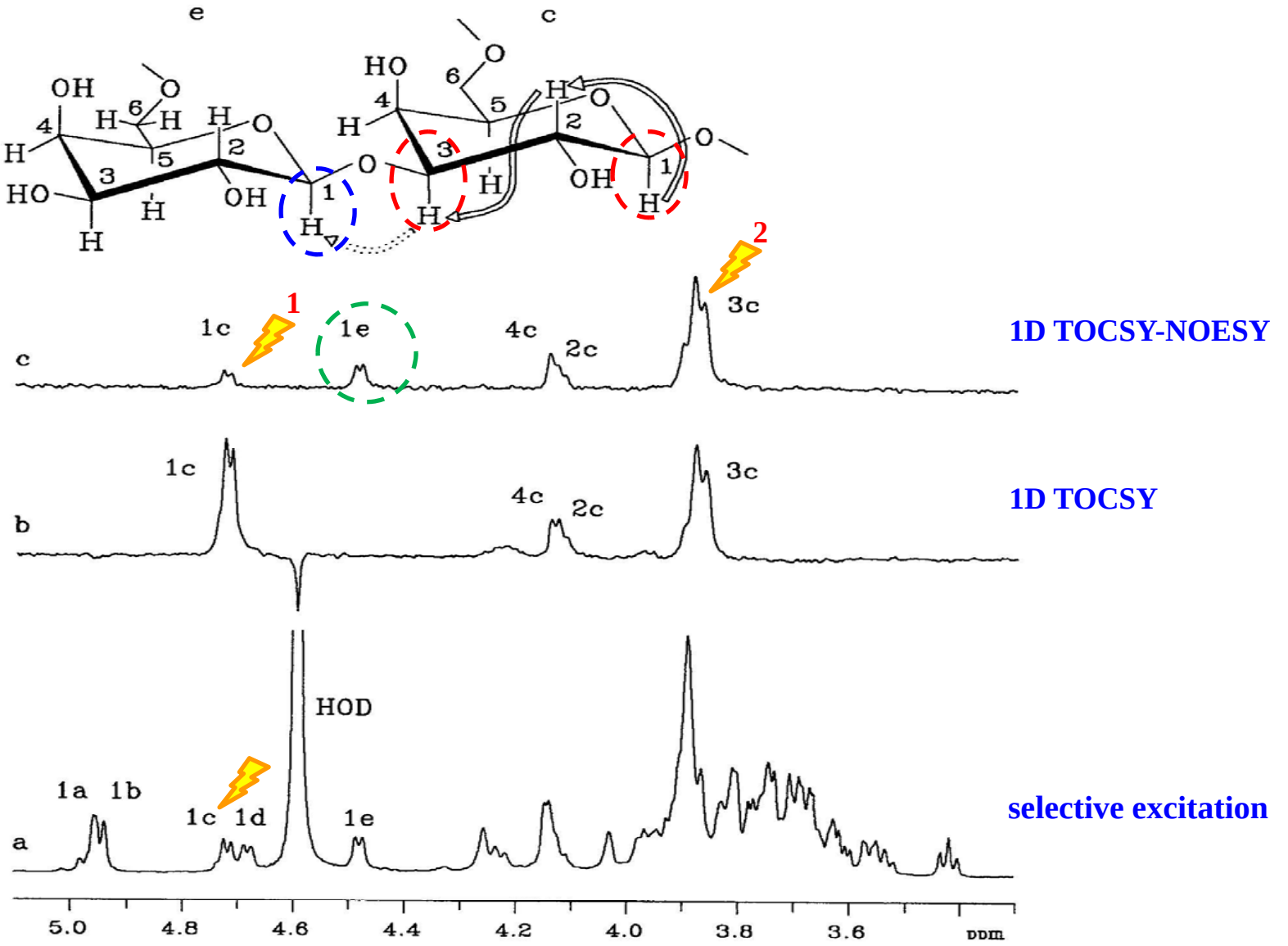


FIG. 5. Illustration of the 1D ge-TOCSY–NOESY technique on polysaccharide **3.** (a) ^{1}H spectrum of **3,** NT = 4; (b) 1D ge-TOCSY spectrum of **3,** NT = 8; H-1c was selectively excited by a 57 ms half-Gaussian pulse, the mixing time was 57.5 ms, and the δ delay was 28.8 ms; (c) 1D ge-TOCSY–NOESY spectrum (NT = 64) of **3** acquired using the pulse sequence of Fig. 1e. Selective pulses preceding the TOCSY and the NOESY periods were half-Gaussian pulses of 57 and 43.5 ms applied to H-1c and H-3c protons, respectively. Mixing times were 57.5 ms for the TOCSY and 250 ms for the NOESY transfer; the δ delay was 28.8 ms . A partial structure of **3** is given in the inset with TOCSY and NOESY pathways indicated by solid and dotted lines, respectively.

Uhrin, D.; Barlow, P. N. *J. Magn. Reson.* ***1997, 126, 248-255.***

7.2

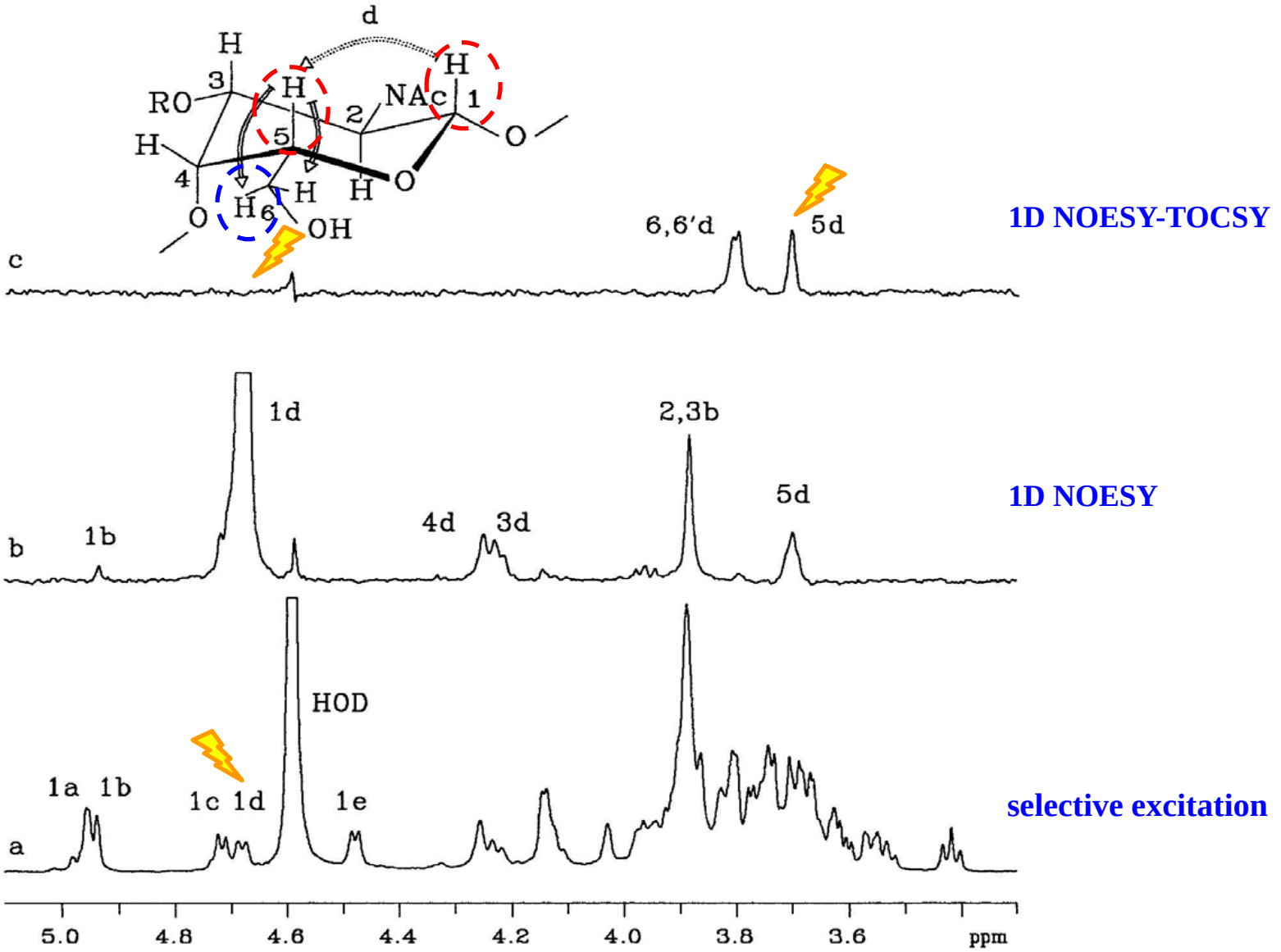


FIG. 6. Illustration of the 1D ge-NOESY–TOCSY technique on compound **3**. (a) ^{1}H spectrum of **3**, NT = 4; (b) 1D ge-NOESY spectrum of **3** (NT = 16); H-1d was selectively excited by a 57 ms half-Gaussian pulse; the mixing time was 250 ms. (c) 1D ge-NOESY–TOCSY spectrum (NT = 128) of **3** acquired using the pulse sequence of Fig. 1f. Selective pulses preceding the TOCSY and the NOESY period were half-Gaussian pulses of 57 and 43.5 ms applied to H-1d and H-5d protons, respectively. Mixing times were 250 ms for the NOESY and 48 ms for the TOCSY transfer; the δ delay was 24 ms. A partial structure of **3** is given in the inset with NOESY and TOCSY transfers indicated by dotted and solid lines, respectively.

Uhrin, D.; Barlow, P. N. *J. Magn. Reson.* ***1997, 126, 248-255.***

Learning NMR

Reference :

- **NMR Guide**
- **The Hebrew University of Jerusalem (http://chem.ch.huji.ac.il/nmr)**
- **Michigan State University (http://www.cem.msu.edu/~reusch/VirtualText/Spectrpy/nmr/nmr1.htm)**
- **Department of Chemistry, Queen's University (http://www.chem.queensu.ca/FACILITIES/NMR/nmr/webcourse/Flash/HX-coupling.swf)**

補1.

核種	自旋量子數	化學位移範圍	標準樣品
$_{1}H$	1/2	15 ~ –2	$Si(CH_3)_4$
$_{6}Li$	1	5 ~ –10	LiCl in H_2O
$_{7}Li$	3/2	5 ~ –10	LiCl in H_2O
$_{13}C$	1/2	250 ~ –20	$Si(CH_3)_4$
$_{15}N$	1/2	1200 ~ –500	CH_3NO_2
$_{17}O$	5/2	1800 ~ –100	H_2O
$_{19}F$	1/2	100 ~ –300	$CFCl_3$
$_{23}Na$	3/2	10 ~ –80	NaCl in H_2O
$_{27}Al$	5/2	300 ~–300	$[Al(H_2O)_6]^{3+}$
$_{29}Si$	1/2	100 ~ –400	$Si(CH_3)_4$
$_{31}P$	1/2	500 ~ –500	H_3PO_4

常用原子核的化學位移範圍值及其標準樣品

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(資料的提供)
(技術的支援)
(知識的分享)